



Dynamic interactions between CO₂ efflux, sap flow and internal CO₂ concentration in tree stems: implications towards the assessment of actual stem respiration

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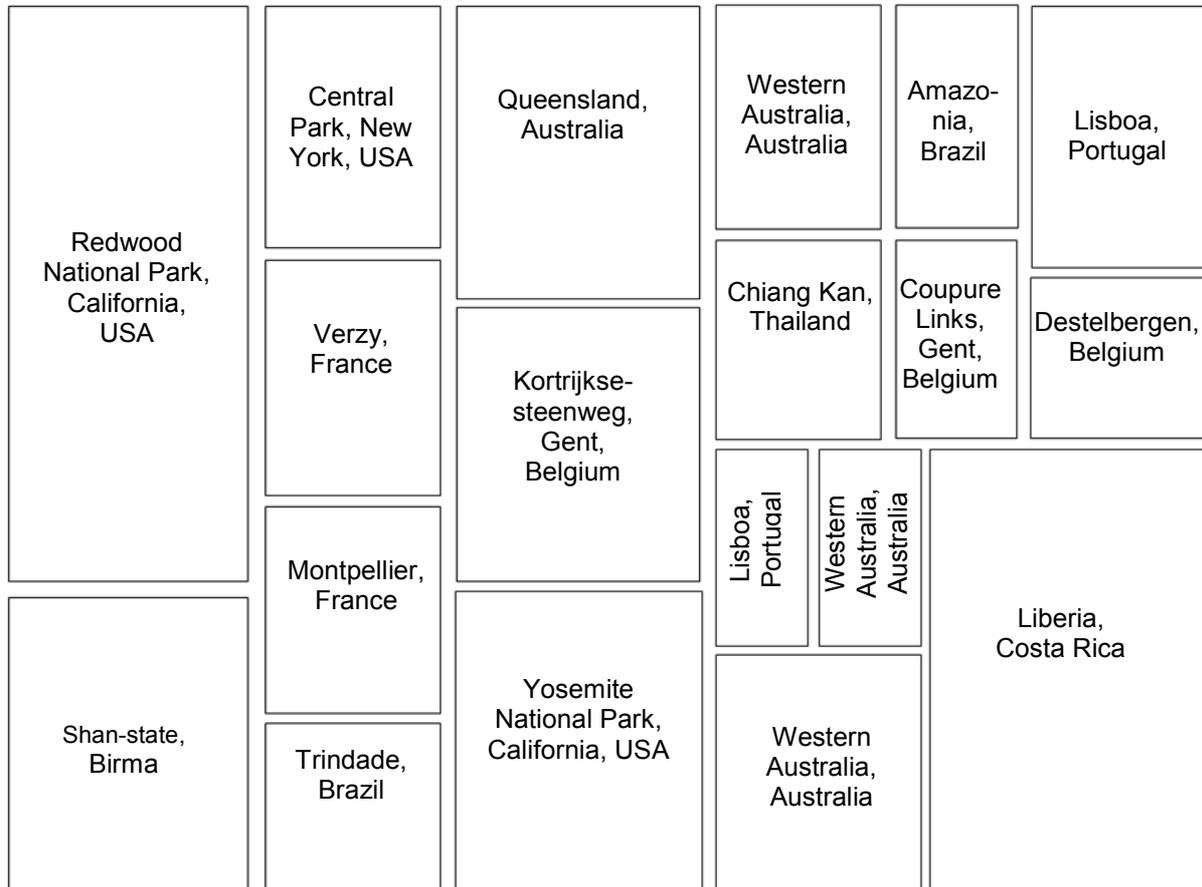
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Dynamic interactions between CO₂ efflux, sap flow and internal CO₂ concentration in tree stems: implications towards the assessment of actual stem respiration

Thesis submitted in fulfillment of the requirements
For the degree of Doctor (PhD) in Applied Biological Sciences

Dynamische interacties tussen CO₂-efflux, sapstroom en interne CO₂-concentratie in boomstammen: implicaties voor de beoordeling van de werkelijke stamrespiratie

Illustrations on the cover:



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List of abbreviations and symbols

Abbreviations

DBH	diameter at breast height
DG	daily growth
DIC	dissolved inorganic carbon
GC	gas chromatograph
IRGA	infrared gas analyzer
LVDT	linear variable displacement transducer
MDS	maximum daily shrinkage
NDIR	non-dispersive infrared
PAR	photosynthetic active radiation
RH	relative humidity
VPD	vapour pressure deficit

Latin symbols

Symbol	Description	Unit
A	stem surface area	(m ²)
A^s	surface area of the virtual membrane separating the stem storage compartment from the xylem compartment	(m ²)
C	capacitance of storage compartment	(mg MPa ⁻¹)
$[CO_2]$	CO ₂ concentration in the gas phase	(%)
$[CO_2](20)$	CO ₂ concentration in the gas phase at 20 °C	(%)
$[CO_2^*]$	DIC concentration in the liquid phase	(mmol l ⁻¹)
$[CO_2^*](20)$	DIC concentration in the liquid phase at 20 °C	(mmol l ⁻¹)
d	thickness of storage compartment	(m)
D	diameter	(m)
D_i	inner diameter of stem segment	(m)
D_o	outer diameter of stem segment	(m)
$\Delta[CO_2]$	difference in CO ₂ concentration in the gas phase	(μ mol mol ⁻¹ or μ mol m ⁻³)

Abbreviations and symbols

$\Delta[\text{CO}_2^*]$	difference in DIC concentration in the liquid phase	($\mu\text{mol l}^{-1}$)
ΔS	storage flux of CO_2	($\mu\text{mol m}^{-3} \text{s}^{-1}$)
e	partial water vapour pressure	(Pa)
e^0	saturated water vapour pressure	(Pa)
E_a	Arrhenius activation energy	(J mol^{-1})
E_{CO_2}	CO_2 efflux rate	($\mu\text{mol m}^{-2} \text{s}^{-1}$ or $\mu\text{mol m}^{-3} \text{s}^{-1}$)
$E_{\text{CO}_2(20)}$	CO_2 efflux rate at 20 °C	($\mu\text{mol m}^{-2} \text{s}^{-1}$ or $\mu\text{mol m}^{-3} \text{s}^{-1}$)
$E_{\text{CO}_2(\text{d})}$	CO_2 efflux rate in the dark	($\mu\text{mol m}^{-2} \text{s}^{-1}$ or $\mu\text{mol m}^{-3} \text{s}^{-1}$)
$E_{\text{CO}_2(\text{l})}$	CO_2 efflux rate in the light	($\mu\text{mol m}^{-2} \text{s}^{-1}$ or $\mu\text{mol m}^{-3} \text{s}^{-1}$)
$E_{\text{H}_2\text{O}}$	H_2O efflux rate (i.e., transpiration)	($\text{mg m}^{-2} \text{s}^{-1}$)
E_{T}	export of dissolved CO_2	($\mu\text{mol m}^{-3} \text{s}^{-1}$)
f	sap flow between a xylem and a storage compartment	(mg s^{-1})
F	sap flow in a xylem compartment	(mg s^{-1})
f_a	air flow rate	(mol s^{-1})
f_s	sap flow rate	(l s^{-1} or g h^{-1})
F_{T}	transport flux of CO_2	($\mu\text{mol m}^{-3} \text{s}^{-1}$)
I_{T}	import of dissolved CO_2	($\mu\text{mol m}^{-3} \text{s}^{-1}$)
k	reaction rate	($\mu\text{mol m}^{-3} \text{s}^{-1}$)
K	acidity constant	(-)
K_{H}	Henry's law constant	(-)
l	length of stem segment	(m)
L	hydraulic conductivity of the virtual membrane separating the stem storage compartment from the xylem compartment	($\text{m MPa}^{-1} \text{s}^{-1}$)
n	amount of gas	(mol)
p	atmospheric pressure	(Pa)
p_{CO_2}	CO_2 partial pressure	(Pa)
Q_{10}	change in rate for a 10 °C change in temperature	(-)
R	diffusion resistance	($\text{m}^2 \text{s mmol}^{-1}$ or s m^{-1})
R_s	respiration rate	($\mu\text{mol m}^{-3} \text{s}^{-1}$)
$R_s(T_r)$	respiration rate at reference temperature T_r	($\mu\text{mol m}^{-3} \text{s}^{-1}$)
R_u	universal gas constant	($\text{J mol}^{-1} \text{K}^{-1}$)
R^s	flow resistance between a xylem and a storage compartment	(MPa s mg^{-1})
R^x	flow resistance in a xylem compartment	(MPa s mg^{-1})
R^2	coefficient of determination	(-)

Δt	time interval	(s)
T	temperature	(°C or K)
T_a	air temperature	(°C)
T_r	reference temperature	(°C)
T_{st}	stem temperature	(°C)
U	potential difference	(V)
v_s	sap velocity	(cm h ⁻¹)
V	sapwood volume of stem segment	(m ³)
V^s	volume of storage compartment	(m ³)
V_w	molar volume of water	(m ³ mol ⁻¹)
w	specific water content of stem segment	(l m ⁻³)
W	water content of storage compartment	(mg)

Greek symbols

ε_0	proportionality constant	(m ⁻¹)
Γ	threshold turgor pressure potential at which wall yielding occurs	(MPa)
Φ	cell wall extensibility	(MPa ⁻¹ s ⁻¹)
ρ_w	density of water	(kg m ⁻³)
Ψ	total water potential of storage compartment	(Pa)
Ψ_p	turgor pressure potential	(Pa)
Ψ_π	osmotic potential	(Pa)
Ψ_{so}	soil water potential	(MPa)
Ψ_{st}	stem water potential	(MPa)
Ψ_{st}^m	midday stem water potential	(MPa)
Ψ_{st}^p	predawn stem water potential	(MPa)
Ψ^x	total water potential of xylem compartment	(MPa)

Introduction and outline of the thesis

“The most conspicuous objective in study of metabolism is measurement of total respiration because it is a measure of the total amount of life”

Woodwell and Botkin (1970)

Studies on the metabolic activity in trees have traditionally focused on leaves as primary indicators of whole-tree metabolism, and most of them have been on the carbon fixation by leaves. Leaf photosynthesis occurs only during the day and in deciduous forests only during the growing season. However, tree metabolism is not restricted to the foliage or to particular periods of the day or year. It occurs continuously in all of the living tissues of a tree. All living cells in a tree expend energy for metabolic processes, and the energy is provided by the respiration process. Total tree (leaf, woody tissue and root) respiration has been estimated to consume up to 78% of gross photosynthesis in forests (Edwards et al. 1981), and is thus a major, even dominant, component of ecosystem carbon budgets. From the perspective of ongoing and predicted climate change, carbon budgets are made worldwide with a focus on forests because of their potential important role as carbon sinks. However, in some cases, the balance between carbon fixed by the forest ecosystem and carbon respired can be very narrow, and forest ecosystems may even act as a source of CO₂. Lindroth et al. (1998) and Valentini et al. (2000) showed that respiration is one of the key factors determining whether forest ecosystems will act as net sources or net sinks of carbon. Stems and branches represent a long-term carbon sink, capable of removing and storing a large amount of atmospheric carbon, but at the same time they lose a considerable amount of carbon in the respiration process to sustain maintenance and growth processes. Although woody tissue respiration had been studied since the end of the 19th century (Müller 1898), it was not until the 1960's and 1970's that its importance was recognized (Stockfors and Linder 1998). In mature forests, respiration from standing woody tissue may equal or exceed leaf respiration on a whole-tree or stand basis (Edwards and Hanson 1996). In a temperate deciduous forest, woody tissue respiration has been reported to represent one third of the total carbon loss by ecosystem respiration (Damesin et al. 2002). Estimations of woody tissue respiration are thus essential in the assessment

of ecosystem carbon budgets. It is therefore surprising that there are relatively few studies on this component of the forest carbon balance.

One important reason for the lack of research on woody tissue respiration relates to the technical difficulties involved in obtaining reliable measurements. Estimates of woody tissue respiration are commonly based on measurements of the efflux of CO₂ from the stem or branch surface into a cuvette. This approach assumes that: (1) the only source of CO₂ efflux is the respiration of local living woody tissues and (2) the only sink of CO₂ produced by the living cells (phloem, cambium and xylem parenchyma) enclosed in the cuvette is radial diffusion to the atmosphere. Ever since cuvettes began to be used to study woody tissue respiration, scientists have been questioning whether the measured CO₂ efflux reflects the actual respiration of the tissue. Researchers have been aware that not all the CO₂ produced by the local living cells may diffuse to the atmosphere (e.g., Boysen-Jensen 1933, Johansson 1933, Maier and Clinton 2006, Wittmann et al. 2006). Moreover, it has been acknowledged that CO₂ present in a tree stem may not only originate from local respiring cells (Levy et al. 1999, Teskey and McGuire 2007). The first chapter of this thesis assembles the information available in literature on the sources and sinks of CO₂ inside tree stems. This chapter further discusses the problems that have been identified in measuring woody tissue respiration rates and the method that has recently been developed (McGuire and Teskey 2004) to quantify the respiration of a stem segment.

Nowadays the scientific community has started to realize that measuring CO₂ efflux rates alone is not sufficient for the quantification of woody tissue respiration. Several other variables are needed to provide us with a better insight into the dynamics of stem respiration and the fluxes of CO₂ inside a stem segment. The second chapter gives an overview of the techniques applied in this thesis for measuring the variables involved in woody tissue respiration research. The chapter gives a brief technical description of the employed sensors and devices and, when alternative measuring techniques were used, both the virtues and shortcomings encountered for each technique are highlighted. It is further evaluated which methods are most appropriate for the application in stem respiration studies. Particular attention is paid to the techniques for measuring stem internal CO₂ concentrations, since some of these techniques have only recently been introduced in tree physiological research.

Temperature is considered an important environmental factor in controlling respiration rates and a simple exponential temperature function is usually applied to predict CO₂ effluxes. However, temperature-independent variations in CO₂ effluxes of woody tissues have been reported several times (Edwards and McLaughlin 1978, Negisi 1972, 1975, 1978, Kakubari 1988, Kaipainen et al 1998). Scientists have claimed that the transpiration stream in the xylem can bias the strong relationship between temperature and stem CO₂ efflux rate. This raises the question how CO₂

efflux rates relate to temperature in the absence of transpiration. To answer this question, the third chapter describes a study conducted on a leafless oak and beech tree during the dormant season. The trees are subjected to controlled temperature and light conditions. Data are presented of stem CO₂ efflux rates and stem internal CO₂ concentrations, the patterns emerging from these observations are discussed, the factors that may contribute to these patterns are determined and the implications for ongoing and future stem respiration studies are summarized.

The transpiration stream has been put forward as a factor affecting CO₂ efflux rates because the flowing sap might be a CO₂ sink, carrying away some of the CO₂ produced by the living tissues enclosed in the cuvette (e.g., Boysen-Jensen 1933, Johansson 1933), or a CO₂ source, releasing CO₂ carried up from the roots and the soil atmosphere at stem level (Levy et al. 1999, Teskey and McGuire 2007). However, another possible cause that has been overlooked is that the transpiration stream also affects the water status in the living stem tissues. Changes in water status may alter rates of growth and maintenance processes and the respiratory processes that support them. In particular, growth may be affected by stem water status as it is one of the most sensitive of all plant processes to drought stress (Hsiao 1973). Chapter 4 describes the case of temperature-independent variations in stem CO₂ efflux rates observed in young beech and oak stems under controlled environmental conditions. The daily dynamics of stem water status and growth rate are assessed and it is determined to which extent these variables are linked to daily variations in stem CO₂ efflux rates. It is hypothesized that the water status of tree stems is a potential important determinant of stem respiration rates as it influences the rate of growth and maintenance processes in the living tissues of the stem. In Chapter 5 the influence of water status on stem respiration rates is further investigated. The water supply of a young oak tree is therefore interrupted for 9 days under controlled environmental conditions. It is examined how the diurnal dynamics of stem CO₂ efflux rates and stem internal CO₂ concentrations change under the gradually developing drought stress and how the relationships between stem CO₂ efflux rates and stem temperature, stem diameter and stem internal CO₂ concentration are affected by the drought stress.

Another major point of discussion among woody tissue respiration studies is the large variability in woody tissue CO₂ efflux rates encountered among and within trees and across stands (e.g., Sprugel 1990, Kaipiainen et al. 1998, Lavigne et al. 1996, Lavigne and Ryan 1997, Damesin et al. 2002). Scaling factors such as sapwood volume, surface area and tissue nitrogen content have only partially succeeded in accounting for the observed variability. Until now, there is still no generally accepted base for expressing CO₂ efflux rates of woody tissues. In Chapter 6, variability in CO₂ stem efflux rates is investigated in a clone of eastern cottonwood trees. The CO₂ concentration of the xylem sap is manipulated under controlled environmental

conditions and the resistance to radial CO₂ diffusion for each tree is determined from the balance between the amount of CO₂ retained in the xylem versus that which diffuses to the atmosphere. The hypothesis is put forward that variability in the diffusion resistance might be an overlooked cause for the large variability in woody tissue CO₂ efflux rates.

Recently, a method has been developed to quantify the respiration rate of a stem segment that accounts for both external (i.e., CO₂ efflux) and internal fluxes of CO₂ in a stem segment (McGuire and Teskey 2004). However, in very few studies the method has actually been applied and, to my knowledge, the relationship between calculated respiration and temperature has never been investigated. In Chapter 7, the respiration rate is calculated using the method of McGuire and Teskey (2004) for stem segments of eastern cottonwood trees under different natural weather conditions. Because the calculated respiration rate accounts for all fluxes of CO₂, and represents the actual respiration rate of the stem tissue, it is hypothesized that it would correlate better with temperature than measured CO₂ efflux. The study further focuses on how stem CO₂ efflux rates and xylem CO₂ concentrations of the trees relate to each other under different natural weather conditions. Gansert and Burgdorf (2005) suggested that measured CO₂ efflux from woody tissues may originate physico-chemically from a strong diffusion gradient between the xylem and the atmosphere rather than from actual respiration. At the other hand, Wittmann et al. (2006) and Maier and Clinton (2006) hypothesized that woody tissue CO₂ efflux mainly represents the respiration of the external tissues (phloem and cambium) because the xylem tissue is isolated as a source of CO₂ efflux due to the much higher respiration rate of the external tissues compared to the xylem. In Chapter 7, it is examined to which extent stem CO₂ efflux rates relate to xylem CO₂ concentrations on sunny and rainy days.

The thesis concludes with a general discussion, summarizing the main findings of the research. Based on these findings, the implications for ongoing and future ecophysiological research are formulated. It is further discussed which questions remain unanswered, and where promising areas of future research lie.

In summary, the following main questions are addressed in this thesis:

- What do we currently know about the different sources and sinks of stem internal CO₂ and what are the concerns about measuring stem respiration?
- Which variables are needed to gain insight into the dynamics of stem respiration and which sensors are suitable for measuring these variables?
- How do stem CO₂ effluxes and xylem CO₂ concentrations relate to temperature in leafless trees, when transpiration is absent?
- Are temperature-independent variations in stem CO₂ efflux related to variations in stem water status?

- How do stem CO₂ efflux and xylem CO₂ concentration change under soil water depletion and how are the relations between stem CO₂ efflux and stem temperature, stem diameter and xylem CO₂ concentration affected by drought stress?
- May variability in CO₂ diffusion resistance contribute to large variations in stem CO₂ effluxes between trees?
- Does calculated stem respiration relate better to stem temperature than stem CO₂ efflux and how do stem CO₂ efflux and xylem CO₂ concentration relate to each other under different weather conditions?

Most of the experiments were conducted in growth chambers under controlled environmental conditions at the Laboratory of Plant Ecology, Ghent University, Belgium and at the School of Forestry and Natural Resources, University of Georgia, USA. The field experiment was performed near Whitehall Forest, Athens, USA. Several tree species were used as study material throughout this thesis. Experiments in Belgium were performed on pedunculate oak, *Quercus robur* L., and on European beech, *Fagus sylvatica* L., two common species in Belgian forests. Experiments in the USA were conducted on eastern cottonwood, *Populus deltoides* Bartr. ex Marsh., a common species throughout the eastern United States.

Chapter 1

On the origin and fate of CO₂ inside tree stems

Adapted from : Teskey RO, Saveyn A, Steppe K and McGuire MA (2007) Tansley Review: the origin, fate and significance of CO₂ in tree stems. *New Phytologist*.(accepted)

1.1 Introduction

Bushong (1907) was the first to analyze the gas extracted from a tree stem. He drew gases from a *Populus deltoides* Bartr. ex Marsh. tree and reported the following percentages: O₂, 1.24; CO₂, 7.21; CH₄, 60.9 and N₂, 30.65. After him, several researchers have studied the gas composition inside tree stems. A general observation with which everyone agrees, is that the composition of gases inside tree stems greatly differs from that in the ambient air. The most remarkable difference with ambient air is the depletion of O₂ and the accumulation of CO₂. O₂ is consumed and CO₂ is produced in the respiration process of the living cells. Unlike animals, plants lack specialized systems for the distribution of O₂ and CO₂. MacDougal (1926) suggested that the supply of O₂ to and the removal of CO₂ from the sites of respiration are restricted because of the large diffusion barriers of the stem tissues. In the remainder of this thesis, the focus will be on CO₂, since the efflux rate of this gas is commonly measured to estimate the respiration rate of woody tissues. It is important to note at this point that CO₂ is not only present in the gaseous phase of the stem, but also in the aqueous phase. Due to the high solubility of CO₂ in water, a portion of the CO₂ dissolves in the xylem sap. The different forms of dissolved inorganic carbon (hereafter abbreviated as DIC) are CO₂ (aq), H₂CO₃, HCO₃⁻ and CO₃²⁻. Henceforth, CO₂ concentrations in the gaseous phase will be indicated as [CO₂] (%) and the total amount of DIC in the aqueous phase as [CO₂*] (mol l⁻¹). Table 1.1 gives an overview of reported values of [CO₂] and [CO₂*] in the xylem of tree stems.

In previous investigations it has been hypothesized that the CO₂ present inside a tree stem may originate not solely from respiration by the living cells, but also from import by the transpiration stream (Levy et al. 1999, Teskey and McGuire 2007). The CO₂ that is imported into the stem originates from root respiration or from soil water taken up by the tree. Stem internal CO₂ may leave the stem in several ways: it may be

released to the atmosphere via radial diffusion, consumed in cortical or wood photosynthesis, or it may be transported by the transpiration stream towards the branches and leaves. In the following paragraphs the different sources and sinks of stem internal CO₂ will be discussed in detail. However, it is important to note that these are only the sources and sinks that have been distinguished in literature. We must realize that this list is probably incomplete.

Table 1.1: Reported values of CO₂ concentrations in the gas phase of the xylem ([CO₂]) or total dissolved inorganic carbon in the xylem sap ([CO₂*]).

Reference	[CO ₂] (%) or [CO ₂ *] (mmol l ⁻¹)	Species
Bushong (1907)	7.2%	<i>Populus deltoides</i> Bartr. ex Marsh.
MacDougal (1927)	1.4 – 18.2%	<i>Populus macdougalii</i> Rose
MacDougal (1927)	1.2 – 15.1%	<i>Quercus agrifolia</i> Nee
MacDougal (1927)	5 – 13.1%	<i>Salix lasiolepis</i> Benth.
MacDougal and Working (1933)	1 – 13.3%	<i>Parkinsonia microphylla</i> Gray
MacDougal and Working (1933)	5 – 22%	<i>Juglans major</i> (Torr.) Heller
MacDougal and Working (1933)	0.04 – 18.5%	<i>Populus tremuloides</i> Michx., <i>Populus macdougalii</i> Rose
MacDougal and Working (1933)	1.1 – 13.5%	<i>Pinus radiata</i> D. Don
MacDougal and Working (1933)	1.4 – 26.3%	<i>Quercus agrifolia</i> Nee.
MacDougal and Working (1933)	4.8 – 13.3%	<i>Salix lasiolepis</i> Benth.
MacDougal and Working (1933)	2 – 9.9%	<i>Sequoia sempervirens</i> (D. Don) Endl.
Chase (1934)	1.73 – 12.25%	<i>Pinus strobus</i> L.
Chase (1934)	1.52 – 16.27%	<i>Ulmus americana</i> L.
Chase (1934)	0.29 – 5.98%	<i>Quercus borealis</i> Michx.
Chase (1934)	0.04 – 2.89%	<i>Quercus macrocarpa</i> Michx.
Chase (1934)	0.15 – 20%	<i>Populus deltoides</i> Bartr. ex Marsh.
Jensen (1967)	13.5 – 16.5%	<i>Quercus rubra</i> L.
Eklund (1990)	~ambient – 10%	<i>Picea abies</i> (L.) Karst.
Eklund (1993)	1 – 5%	<i>Acer platanoides</i> L.
Eklund (1993)	1 – 9%	<i>Quercus robur</i> L.
Hari et al. (1991)	0.3 – 2%	<i>Pinus sylvestris</i> L.
Stringer and Kimmerer (1993)	0.5 – 0.9 mmol l ⁻¹	<i>Populus deltoides</i> Bartr. ex Marsh.
Kaipainen et al. (1998)	0.27 – 2.5%	<i>Pinus sylvestris</i> L.
Levy et al. (1999)	3 – 9.2%	<i>Betula pendula</i> Roth
Levy et al. (1999)	3.02 mmol l ⁻¹	
Levy et al. (1999)	3.2%	<i>Distemonanthus benthamianus</i> Baill.
Levy et al. (1999)	1.2 mmol l ⁻¹	
Levy et al. (1999)	8.5%	<i>Musanga cecropioides</i> R. Br. ex Tedlie
Ceschia (2001)	10.98 mmol l ⁻¹	
Teskey and McGuire (2002)	1%	<i>Picea abies</i> (L.) Karst.
Teskey and McGuire (2002)	0.7 – 18.7%	<i>Liriodendron tulipifera</i> L., <i>Quercus alba</i> L.
McGuire and Teskey (2004)	1.6 – 10.3 mmol l ⁻¹	
McGuire and Teskey (2004)	2 – 4.5 mmol l ⁻¹	<i>Fagus grandifolia</i> Ehrh.
McGuire and Teskey (2004)	1.8 – 3 mmol l ⁻¹	<i>Liquidambar styraciflua</i> L.
McGuire and Teskey (2004)	1.7 – 3.5 mmol l ⁻¹	<i>Platanus occidentalis</i> L.
Maier and Clinton (2006)	1 – 8%	<i>Pinus taeda</i> L.
Teskey and McGuire (2007)	5.6 – 12.0%	<i>Platanus occidentalis</i> L.
	1.8 – 3.5 mmol l ⁻¹	

1.2 Sources of stem internal CO₂

1.2.1 Soil water

[CO₂] in the soil is much higher than that of the ambient air. The sources of CO₂ in the soil are respiration of the roots and their symbiotic mycorrhizal fungi and of the free-living microbial and faunal populations of the soil. Soil [CO₂] can range from near atmospheric levels to as high as 130000 ppm (Amundson and Davidson 1990). CO₂ in the gas phase of the soil is in equilibrium with DIC in the soil water. Several studies have shown that DIC can be absorbed by the roots (Livingston and Beall 1934, Stolwijk and Thimann 1957, Bedri et al. 1960, Skok et al. 1962, Arteca and Poovaiah 1982, Amiro and Ewing 1992). Hence, the xylem sap contains an amount of DIC originating from the soil. DIC may be further transported with the transpiration stream towards the stem, branches and leaves. Indirect evidence for the importance of soil DIC is given in the studies of Vuorinen et al. (1989, 1995). In the first study (1989) NaHCO₃ was added to the water bathing the roots of *Salix sp.* cuttings and it was found that growth was increased by up to 31%. In the second study (1995) it was found that the biomass production of *Betula pendula* Roth trees grown in NaHCO₃ enriched hydroponic culture media increased by ca 10%. Both studies indicate that DIC was most probably taken up by the roots and assimilated in photosynthesis. Hibberd and Quick (2002) provided direct evidence for uptake, transport and photosynthetic fixation of soil DIC. They found that DIC supplied as [¹⁴C]NaHCO₃ to the roots of tobacco grown hydroponically led to the accumulation of ¹⁴C in insoluble material, most notably starch, in cells associated with the vascular system. We are unaware of any studies on the influence of soil DIC on xylem sap [CO₂*] in the stem, but it has been suggested that this influence might be of great importance. Levy et al. (1999) found that sap flow rate and stem CO₂ efflux rate of the shrub *Combretum micranthum* G. Don were positively related. They attributed this positive correlation to the uptake and transport of water containing large amounts of DIC. When the water is drawn into the stem, where [CO₂*] may be lower, CO₂ is released from the xylem sap and contributes to an extra radial diffusion of CO₂ out of the stem. However, soil [CO₂*] and xylem [CO₂*] were not measured in this experiment. Teskey and McGuire (2007) did measure soil [CO₂] and xylem [CO₂] at the stem base of *Platanus occidentalis* L. trees. On the average, soil [CO₂] was 1.2%, whereas xylem [CO₂] at the stem base was 7.6%, i.e., 6 times higher. Therefore, they concluded that only a small portion of the CO₂ in the xylem could have originated from soil water.

1.2.2 Root respiration

The sap coming from the soil is further enriched with CO₂ produced by the living cells in the roots. Roots in the soil are exposed to a high [CO₂] environment, which hinders radial diffusion of CO₂ from the roots to the soil atmosphere (Qi et al. 1994). Moreover, physical barriers to CO₂ diffusion in roots are high. This causes a build-up

of CO₂ inside the roots. High [CO₂] in roots has been reported previously (Clements 1921, Rakonczay et al. 1997). Recently, Teskey and McGuire (2007) demonstrated that a substantial portion of the CO₂ within tree stems originated from the root system. Therefore, they hypothesized that larger trees with larger root systems may accumulate more CO₂ in the xylem sap.

1.2.3 Stem respiration

The main source of CO₂ in tree stems is the respiration of living cells in the stem. Although the stem cross-section consists mainly of dead xylem vessels, phloem parenchyma, cambium and ray parenchyma in the xylem are living (Figure 1.1). The respiration rate (R_S) depends on the amount of living cells and on the rate of metabolic activity of the living cells.

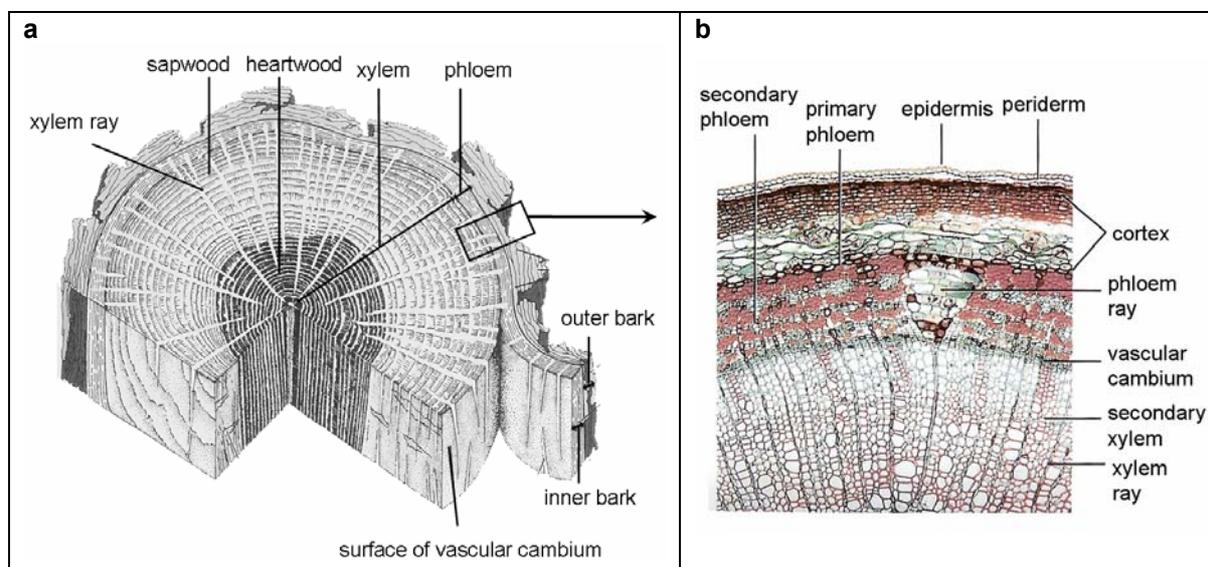


Figure 1.1: Anatomical structure of a tree stem (adapted from Raven et al. 1992).

1.2.3.1 Amount of living cells

The phloem and cambium consist mainly of living cells, whereas in the xylem only the parenchyma ray cells are living. Stockfors and Linder (1998) found that xylem living cell volume in *Picea abies* (L.) Karst. was only 0.89% of total xylem volume, while phloem living cell volume was 20.9% of total phloem volume. However, in large stems, the ratio between inner bark (i.e., phloem and cambium) volume and xylem volume becomes smaller and the amount of living cells in the xylem may surpass that of the inner bark. Ryan (1990) found that for *Pinus contorta* var. *latifolia* Engelm. and *Picea engelmannii* Parry trees with diameter at breast height (DBH) ranging from 4 - 40 cm, the xylem contained more than 80% of the total living cell volume and the fraction of living cells in the xylem remained relatively constant with distance from the cambium. Ceschia et al. (2002) found that in the trunk of *Fagus sylvatica* L. with a mean DBH of 7.2 cm the amount of living cells in the xylem surpassed that of the inner bark. The fraction of living cells in the xylem remained constant with distance

from the cambium. Contrarily, Stockfors and Linder (1998) found that in *Picea abies* (L.) Karst. trees with DBH ranging from 6.5 – 10.2 cm, the xylem contained only 20% of total living cell volume and the fraction of living cells in the xylem decreased exponentially with distance from the cambium.

To investigate the major respiratory source of CO₂ inside stems, several authors have measured CO₂ efflux rates (E_{CO_2}) from different tissue types of cut stem segments. Zabuga and Zabuga (1990) measured CO₂ exchange of different stem tissues of *Pinus sylvestris* L. trees in the growing season. Stem segments were divided in inner bark (phloem and cambium), outer sapwood and inner sapwood. They found that E_{CO_2} of the cambium and phloem tissues was higher than that of the sapwood and that E_{CO_2} of inner and outer sapwood were similar. Pruyn et al. (2002a) took increment cores of *Pseudotsuga menziesii* (Mirb.) stems in the dormant season and cut them into four segments: inner bark, outer, middle and inner sapwood. They found that E_{CO_2} of inner bark was 2 – 3 times higher than that of sapwood and E_{CO_2} of outer sapwood was 50 – 70% higher than that of inner sapwood. They did similar research during the dormant season on *Pinus ponderosa* Dougl. Ex Laws. stems (Pruyn et al. 2002b) and found that E_{CO_2} of inner bark was 3 – 15 times greater than that of sapwood, and E_{CO_2} of outer sapwood was 30 – 60% higher than that of middle or inner sapwood. Bowman et al. (2005) took increment cores from *Dacrydium cupressinum* Lamb stems during the growing season and cut them into 7 segments: inner bark, outer and inner sapwood, sapwood-heartwood boundary, and outer, middle and inner heartwood. The O₂ consumption (which served as an estimation of the respiratory activity) of the inner bark samples was significantly higher than that of other tissue types. The O₂ consumption rapidly declined with depth into the stem. Hence, in all studies the inner bark was found to be the most metabolically active tissue. This is in correspondence with the larger phloem living cell volume / total phloem volume compared to the xylem living cell volume / total xylem volume as found by Stockfors and Linder (1998).

1.2.3.2 Rate of metabolic activity of living cells

(1) Maintenance and growth metabolism

Several processes in tree stems require energy, such as amino-acid synthesis, protein synthesis, structural dry matter synthesis and ion membrane transport (Dewar 2000). Some of these processes take place in every living cell during every season. R_S associated with these active processes is the so-called maintenance R_S (Penning de Vries 1975). Other energy-requiring processes take only place during a particular period of the year and in particular tissues. For example, during spring, when environmental conditions become favourable, R_S of the cambial layers increases because much energy is needed for cell division and differentiation of the cells into xylem and phloem elements. Energy is also required for cell wall deposition and assembly of newly formed cells. In the early stages of shoot elongation, energy is

needed for the loading of carbohydrates from storage locations in the xylem parenchyma into the phloem (Ryan 1990). During the growing season, R_S of phloem cells is also high because energy is needed for the loading of photosynthates into storage locations in the stem. The respiration associated with these processes is the so-called growth respiration (Penning de Vries et al. 1974) and is mainly associated with the cambial layers and the phloem. Hence, R_S of woody tissues, particularly of external tissues (cambium and phloem), is higher during the growing season than during the dormant season due to the large energy requirements of growth related processes.

(2) Influence of temperature

Temperature affects metabolic processes by way of its influence on the reaction kinetics of chemical events and on the activities of the various enzymes involved. The increase of a reaction rate as a result of a temperature increase can be described by the Arrhenius formula:

$$k = a \times \exp\left(-\frac{E_a}{R_u \times T}\right) \quad (1.1)$$

where k is the reaction rate at temperature T (K), a is a constant, E_a is the Arrhenius activation energy for a given reaction and R_u is the universal gas constant ($8.314 \text{ J K}^{-1} \text{ mol}^{-1}$) (Thornley and Johnson 1990). Environmental physiologists often write the relationship between respiration rate and temperature as:

$$R_S = R_S(T_r) \times Q_{10}^{\frac{T-T_r}{10}} \quad (1.2)$$

where R_S is the respiration rate at temperature T , $R_S(T_r)$ the respiration rate at reference temperature T_r and Q_{10} the increase in respiration rate that results from a temperature increase of 10°C (Amthor 1989, Ryan 1990, Ryan et al. 1994, 1995, Carey et al. 1996, Levy and Jarvis 1998, Maier et al. 1998, Stockfors and Linder 1998, Levy et al. 1999, Xu et al. 2000, Meir and Grace 2002, Damesin 2003, Gielen et al. 2003, Wang et al. 2003). This empirical approach is based on the observation that, over a restricted temperature range, which is usually of interest to the biologist, a given temperature increment increases the reaction rate by a constant factor. However, when the influence of temperature over a wide range is of interest, it should be kept in mind that the Q_{10} of an enzymatic reaction is not constant. At low temperatures Q_{10} is large since enzymatic reactions are rate-limiting, whereas at higher temperatures Q_{10} becomes smaller because physical processes such as diffusion velocity become limiting (Larcher 2003). At very high temperatures, reactions take place so rapidly that substrate (source) and metabolites (e.g., ADP) (sinks) can not keep up with the high turnover rate of substance and energy, so that

the respiration rate soon drops (Figure 1.2). The Q_{10} is typically about 3 at low temperature and becomes less than unity at very high temperatures (Amthor 1994).

However, in nature, this decline in respiration is seldom reached, so that a respiration rate increase with any increase in temperature is likely to be experienced by a plant (Amthor 1994). For a wide variety of plant materials (mostly agricultural crops) Q_{10} ranges from 1.6 to 3, but centers about 2 in the physiologically relevant temperature range (Amthor 1989). Q_{10} reported for woody plants appears to vary less, but might be slightly higher (Ryan 1991b). However, it should be noted at this point that estimates of R_S are usually based on E_{CO_2} measurements, assuming that E_{CO_2} equals R_S . At the end of this review it will hopefully become clear to the readers that this approach is incorrect, implying that estimates of Q_{10} may also be erroneous.

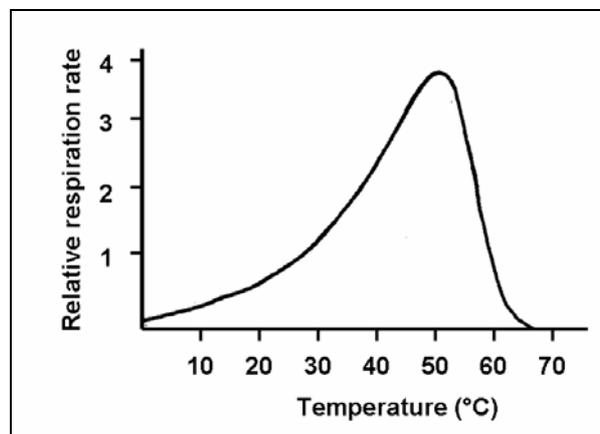


Figure 1.2: An idealized relationship between respiration rate and tissue temperature. (Note: this curve applies to short-term changes in temperature; long-term changes in temperature can induce acclimatization) (adapted from Amthor 1994).

(3) Influence of nutrient status

Nutrient status, especially nitrogen concentration ($[N]$), is likely to affect R_S , because typically 90% of the nitrogen in plant cells is in protein, which needs energy for maintenance processes such as replacement and repair (Penning de Vries 1975). However, evidence for a strong relationship between tissue $[N]$ and maintenance R_S in woody tissues is lacking. Vose and Ryan (2002) found that dormant season stem E_{CO_2} per unit mass was correlated with $[N]$ in *Pinus strobus* L. trees, but Lavigne and Ryan (1997) found no relationship between maintenance E_{CO_2} and sapwood $[N]$ in several boreal tree species. Experimental data examining effects of artificially altering soil nitrogen availability on stem E_{CO_2} are scarce. Nitrogen fertilization may affect stem R_S by increasing tissue $[N]$ and the associated protein turnover costs. Maier et al. (1998) reported that fertilized *Pinus taeda* L. trees had increased dormant season stem E_{CO_2} per unit sapwood volume compared with controls. In their study, total E_{CO_2} was linearly correlated with tissue $[N]$. Contrarily, Stockfors and Linder (1998) measured no effect of stem $[N]$ on stem E_{CO_2} per unit live cell volume in fertilized *Picea abies* (L.) Karst. trees. Ryan et al. (1996) also found little effect of fertilization

on stem maintenance E_{CO_2} per unit sapwood volume in *Pinus radiata* D. Don. trees. In addition, growth R_S should increase with nitrogen availability, if only because growth rate increases with nitrogen supply (Maier 2001). However, it appears that increased growth E_{CO_2} under fertilization results merely from an increased growth rate, and is not affected by changes in tissue [N] (Stockfors and Linder 1998, Maier 2001). In conclusion, the effect of nitrogen availability on woody tissue R_S is still poorly understood. However, it should be noted that stem E_{CO_2} does not necessarily equals stem R_S , so that the conclusions of these studies may also be incorrect. This issue will be discussed more in detail at the end of this review.

(4) Influence of water status

Also the water status may affect R_S of woody tissues. McCree (1986) reviewed effects of soil water depletion on plants from the perspective of the carbon balance. An important point is that a slowly developing drought stress, as occurs in the field during drought, reduces photosynthesis and growth. Particularly growth processes such as cell expansion and cell wall synthesis are reduced by water deficit as they are one of the most sensitive to drought stress among all plant processes examined (Hsiao 1973). Lockhart (1965) stated that cell expansion is directly proportional to the cell turgor pressure, which is an indicator of the water status of the cell. Besides cell expansion, also cell wall assembly and deposition has been reported to be controlled by cell turgor pressure (Proseus and Boyer 2006). Hence, growth R_S is expected to decrease under soil water depletion. On the other hand, also maintenance R_S is expected to decline under drought stress, due to an overall slowing of metabolic activity (Amthor and McCree 1990, Ryan 1991b).

However, not only soil water depletion may cause drought stress. Water reserves of the stem are depleted during the daytime as a consequence of sap flow. When water reserves are depleted, the living stem tissues may suffer from water deficit, which temporarily reduces rates of growth and maintenance processes during the daytime. Differences in plant growth rate during day and night have been reported by several authors. Boyer (1968) measured the enlargement of leaves of a well-watered sunflower plant (*Helianthus annuus* L.) during day and night and observed that the leaf growth was 5 to 6 times higher at night than during the day. Also Schurr et al. (2000) found that leaf growth rates in *Ricinus communis* L. plants peaked during the late night and reached a minimum in the late afternoon. Halter et al. (1996) observed that root elongation rates in *Eucalyptus nitens* and *E. pauciflora* seedlings were 60 and 67% higher, respectively, during the night than during the day. Solari et al. (2006) found that the shoot extension growth rate of *Prunus persica* (L.) Batsch trees was lower during the midday than in early morning. As a consequence, the energy demand to sustain the growth processes and, hence, R_S is expected to be higher at night. However, to our knowledge, it has not been investigated yet whether stem R_S is actually affected by drought stress.

(5) Influence of oxygen availability

There has long been speculation that aerobic R_S of phloem, cambium and wood parenchyma may become restricted by insufficient O₂ supply. Kimmerer and Stringer (1988) found that ethanol, a characteristic product of anaerobic R_S , was present in the vascular cambium of several tree species, indicating that R_S in this tissue may be O₂ limited. Van Dongen et al (2003) found that O₂ levels in the phloem of *Ricinus communis* L. stems were as low as 7%, i.e., 3 times lower than the atmospheric O₂ concentration ([O₂]). They demonstrated that this level is in the range where energy metabolism is limited and where phloem transport starts to become limited. When the O₂ concentration was further lowered artificially, phloem function was severely inhibited. Reported values for [O₂] in the xylem range from 1% to 19% (Eklund 2000, del Hierro et al. 2002) with variations due to differences in time of the year, species and ages and position within the stem. Spicer and Holbrook (2005) found that in four temperate species that differ widely in anatomy and sapwood longevity, average xylem [O₂] was about 10%, with the lowest values observed in the innermost sapwood around 3-5%. Artificially reducing [O₂] to a level present in the innermost sapwood had a minor effect on the O₂ consumption rate (as a measure of R_S) and further lowering [O₂] to extreme low levels, whose occurrence is likely rare and transient, only moderately limited O₂ consumption. They concluded that respiration of xylem parenchyma is unlikely inhibited at O₂ levels common within stems. Hence, a consensus about whether or not O₂ limits R_S in the stem living tissues has not been achieved so far, but it appears to be tissue-dependent.

(6) Influence of carbohydrate availability

Very few studies have examined the role of carbohydrate availability on woody tissue metabolism and they give rather weak indications about the effect on stem R_S . Martin et al. (1994) reduced the carbohydrate supply of *Pinus taeda* L. seedlings by changing leaf photosynthetic rates and found that increasing photosynthetic rates caused small, gradual reductions in stem E_{CO_2} . However, reducing photosynthetic rates had no significant effect on stem E_{CO_2} . They concluded that additional investigations were needed to clarify the relationship between carbohydrate availability and stem R_S . Daudet et al. (2005) found that stem E_{CO_2} of potted walnut trees gradually increased under a prolonged light period of 8 days. They suggested that stem metabolism increased due to an increased carbohydrate content as a consequence of increased photosynthesis. Another technique that has been applied to investigate the effect of carbohydrate availability on stem R_S is physical girdling (i.e., removing a band of phloem from the stem) and measuring E_{CO_2} above and below the girdle. Edwards and McLaughlin (1978) found that girdling the stem of a *Liriodendron tulipifera* L. tree resulted in an increase of stem E_{CO_2} above the girdle up to 5 times higher than the control, while E_{CO_2} below the girdle dropped to about one third of the control. Martin et al. (1994) found that stem E_{CO_2} of *Pinus taeda* L. seedlings measured below a girdling slowly decreased. Also Ogawa (2006) found

that stem E_{CO_2} from *Pinus sylvestris* L. trees subjected to a girdling treatment gradually declined. However, girdling is a mechanical injury. Teskey and McGuire (2005) demonstrated that by wounding a tree, CO_2 efflux at the place of wounding increased sharply because the barriers for CO_2 diffusion (i.e., the bark) are removed. Therefore, interpretations of the girdling experiments should be treated with caution: the observed reduction in E_{CO_2} in the girdling experiments might be rather the effect of diminishing the barriers for CO_2 diffusion than the effect of interrupting substrate supply. Johnsen et al. (2007) girdled *Pinus taeda* L. trees both physically and physiologically, the latter by chilling the phloem. They found that stem E_{CO_2} was only slightly higher above the girdling than below in the physiologically girdled trees, whereas it was much higher in the physically girdled trees. Hence, when the bark was not removed, the effect on stem E_{CO_2} was much less pronounced.

1.3 Sinks of stem internal CO_2

At least three sinks for stem internal CO_2 have been distinguished in literature: (1) radial molecular mass flow of CO_2 out of the stem into the atmosphere (i.e., CO_2 efflux), (2) re-fixation of CO_2 by corticular and/or wood photosynthesis and (3) export of dissolved CO_2 with the transpiration stream. A schematic of these processes is shown in Figure 1.3.

1.3.1 Diffusion of CO_2 out of the stem (= CO_2 efflux)

CO_2 produced by the living cells of inner bark, cambium and xylem (Figure 1.3, arrows 1a, 1b and 1c, respectively) or CO_2 imported by the transpiration stream (arrow 1d) can be radially transported via molecular mass flow (i.e., diffusion), either in the gaseous phase or in the aqueous phase of the stem. The rate of diffusion in both media depends on temperature because temperature determines the rate at which individual molecules move. Other factors that affect diffusion rates are discussed in the paragraphs below.

1.3.1.1 Diffusion in the gaseous phase

Stem internal gaseous CO_2 diffuses through inter- and intracellular gas spaces out of the stem into the atmosphere following concentration gradients characterized by Fick's law of diffusion:

$$E_{\text{CO}_2} = \frac{\Delta[\text{CO}_2]}{R} \quad (1.3)$$

where E_{CO_2} is the flux of CO_2 out of or into the stem, $\Delta[\text{CO}_2]$ is the difference in CO_2 concentration between the gas spaces of the stem and the atmosphere and R is the sum of a series of diffusion resistances. The sum of resistances that has to be accounted for depends on where the CO_2 is located: in the phloem or in the xylem.

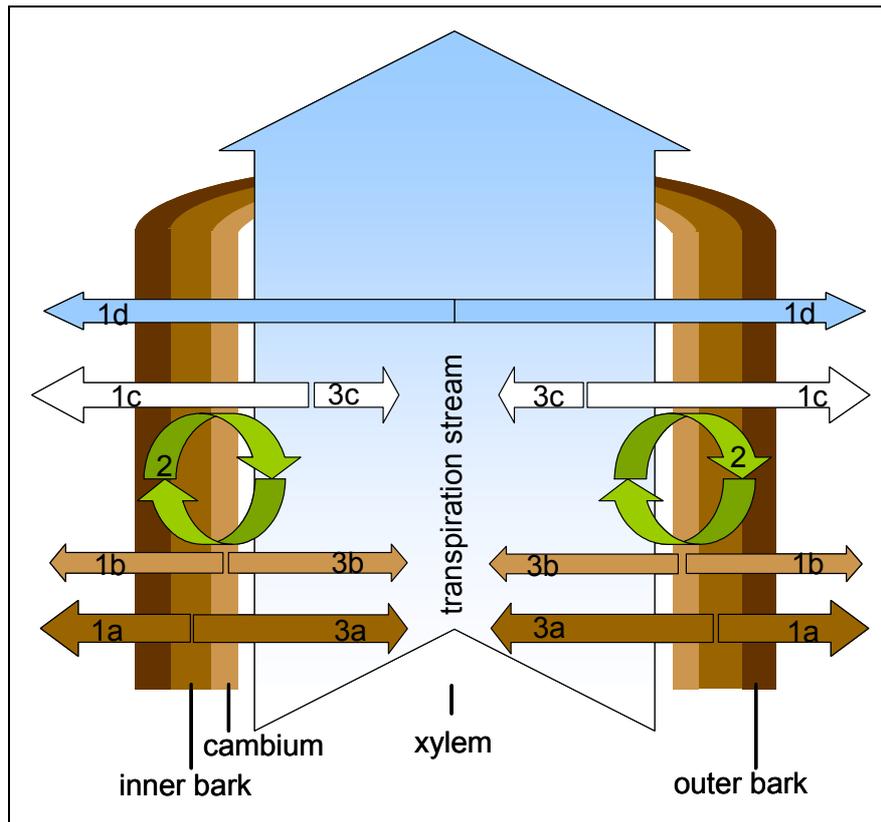


Figure 1.3: Schematic of important sinks of CO₂ inside a tree stem segment. Numbers 1 indicate radial CO₂ diffusion out of the stem, numbers 2 re-fixation of CO₂ by cortical photosynthesis and numbers 3 transport of dissolved CO₂ with the transpiration stream. Letters a indicate fluxes from inner bark, letters b fluxes from cambium, letters c fluxes from xylem and letters d fluxes from the transpiration stream.

(1) CO₂ located in the phloem

For CO₂ located in the phloem, resistances are mainly determined by the cortex (i.e., the tissue between the phloem and the periderm, Figure 1.1), the peridermal layers (phelloderm, phellem (i.e., cork) and phellogen (i.e., cork cambium)) and the rhytidome (i.e., outer bark) (Ziegler 1957). These tissues protect the tree against water loss, at the same time, however, limiting the exchange of CO₂ and O₂ with the atmosphere. The resistance depends mainly on the chemical constituents (lignins, suberins, lipids and waxes) and on thickness (Schönherr 1982). Lenzian (2006) found that in isolated phellem of several tree species thickness and H₂O permeability were fairly well correlated. Pilarski (1994) measured the diffusion conductance (=1/R) of the bark of *Syringa vulgaris* L. stems of different age. The measurements were conducted for H₂O and recalculated for CO₂. CO₂ conductances for current- (thinnest bark), one- and three- (thickest bark) year-old stems were 33, 18 and 10 mmol m⁻² s⁻¹, respectively. Cernusak and Marshall (2000) found that mean CO₂ conductance of the bark surface of branches of *Pinus monticola* Dougl. ex D. Don (mean diameter 1.68 cm) was 0.68 mmol m⁻² s⁻¹. Wittmann et al. (2006) found that mean CO₂ conductance of the bark surface of 6-year-old *Betula pendula* Roth trees was 0.50 mmol m⁻² s⁻¹.

CO₂ diffusion is facilitated by lenticels, cracks and wounds (Grosse 1997, Langenfeld-Heyser 1997) (Figure 1.4). Groh et al. (2002) found that single lenticels from *Betula potaninii* L.C. Hue and *Sambucus nigra* L. were significantly more permeable to O₂ than phellem areas without lenticels. However, data on CO₂ permeability are lacking. Groh et al. (2002) also demonstrated that the O₂ permeability of lenticels changed seasonally. The passage of O₂ was minimal in autumn and winter. In spring, permeability increased to a maximum, which lasted from June to August. Hence, gas exchange is higher during the growing season. A possible explanation for the seasonal changes is based on the fact that during spring, with the formation of new filling cells (Figure 1.4), the closing layers of the lenticels become separated and middle lamellae are partly destroyed, leaving the tissue outside the phellogen with a continuum of intercellular spaces (Lendzian 2006).

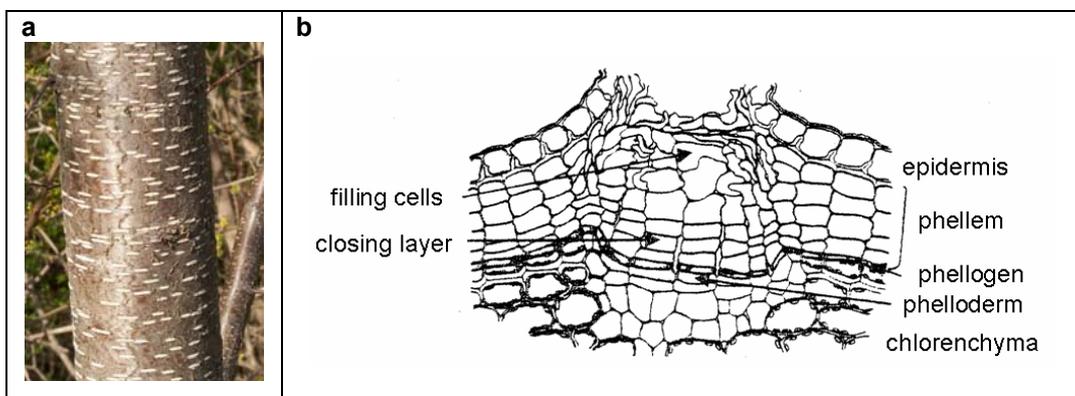


Figure 1.4: (a) Numerous lenticels in the bark of *Prunus serotina* Ehrh.; (b) anatomical structure of lenticellular tissues (adapted from Braune et al. 1991).

The tissues of the phloem itself are chiefly aerated by numerous gas spaces, which have continuity with the external environment (Hook et al. 1972). However, to our knowledge, measurements of phloem permeability have never been conducted.

(2) CO₂ located in the xylem

For CO₂ located in the xylem, the cambium forms a severe barrier for free gas exchange. Gas movement through the cambium has been investigated by MacDougal (1932). He found that the cambium of *Quercus agrifolia* Nee showed no microscopic openings through which gases might pass by streaming, but he found that such passage was possible when a minimum pressure was applied. In some tree species adapted to waterlogged soil, the cambium may contain small intercellular spaces permitting radial CO₂ diffusion (Hook and Brown 1972, Buchel and Grosse 1990). In most species the cambium significantly inhibits lateral gas movement (Kramer and Kozlowski 1979). This explains the large difference in [CO₂] between xylem and bark tissues: values for xylem [CO₂] have been reported to be as high as 26% (Table 1.1), whereas published values of [CO₂] in bark tissues range between 0.06 and 0.17% (Cernusak and Marshall 2000, Wittmann et al. 2006). The permeability of the cambium may also change seasonally. Joseph and Kelsey (2004)

found that when aerobic respiration was stimulated in stem segments of *Pseudotsuga menziesii* (Mirb.) Franco, ethanol production was not induced in segments sampled in May, while it was induced in segments sampled in December. Ethanol production is associated with O₂ limited conditions. They reasoned that actively dividing cambium cells and newly generated xylem and phloem cells in May have thin cell walls that are likely to exchange CO₂ and O₂ more efficiently than cells in December, which are winter-hardened, i.e., protoplasmic viscosity has increased and cell walls are thickened. Also Stockfors and Linder (1998) provided evidence for seasonal changes in tissue permeability for *Picea abies* (L.) Karst, by recording the time lag between changes in stem temperature and subsequent changes in stem E_{CO_2} . The shortest lag was observed in June and it increased in duration through the summer to a maximum in September. Therefore, they assumed that the diffusion resistance was lowest in June and highest in September.

However, the xylem itself may be a greater barrier to free gas exchange than the cambium per se. Sorz and Hietz (2006) found that minimum diffusion coefficients for O₂ in water saturated xylem were always lower than the diffusion coefficients in water alone, illustrating that the xylem cell walls present a major barrier to gas diffusion. However, they could not find a relationship between the maximum diffusion coefficient measured at minimum water content, when diffusion should largely be limited by cell walls, and the volumetric proportion of the cell walls. They therefore hypothesized that the arrangement of cell walls and the structure of the cell wall matrix and/or the pit membranes have a stronger impact on gas exchange than the amount of cell walls. This hypothesis is supported by the estimation of Gartner et al. (2004) that the gas in the xylem is mainly located in the pore spaces of the cell walls and in the lumens of vessels, tracheids or fibres, and not in the intercellular spaces.

1.3.1.2 Diffusion in the aqueous phase

CO₂ is also transported in the aqueous phase across living cells via diffusion. However, the diffusion coefficient of CO₂ in air is $1.6 \times 10^{-5} \text{ m}^2 \text{ s}^{-1}$, whereas it is only $1.6 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$ in water at 20 °C and 101.3 kPa (Nobel 1999), so the diffusion of CO₂ through gas spaces will be much more efficient than in the aqueous phase. Sorz and Hietz (2006) measured radial diffusion of O₂ against N₂ in wood of different trees at different water and gas contents and found that the diffusion coefficient was strongly related to the gas content: at 40% gas volume, the diffusion coefficient increased 5-13 fold in *Picea abies* (L.) Karst, *Taxus baccata* L. and *Quercus robur* L., 36-fold in *Fraxinus excelsior* L. and about 1000-fold in *Carpinus betulus* L. and *Fagus sylvatica* L. compared to the diffusion coefficient at 15% gas volume. Since diffusion in air is much more efficient than in water, the diurnal and seasonal variation in the ratio gas/water content in wood, cambium and periderm has likely an important influence on radial CO₂ diffusion rates. This was noticed by Hook et al. (1972), who suggested that the diurnal shrinkage and swelling of stem tissues, corresponding to

diurnal cycles of changes in stem water content, may influence the diffusion rate of gases into and out of stems. Under periods of stem shrinkage, liquid water films from gas spaces are lost and the stem tissue may become more permeable to gas exchange. The ratio gas/water content in tree stems not only varies diurnally, but also seasonally. Pausch et al. (2000) found that the volumetric water content of the sapwood of *Acer saccharum* L. trees changes significantly during the year and can range from nearly 50% during winter and early spring, to 20% during the growing season. MacDougal et al. (1929) stated that in summer the gas content is high because of the high water loss due to transpiration and because of the large gas production due to respiration. During spring and autumn water is most abundant and the gas volume is greatly reduced. This seasonal change is likely to be most pronounced in deciduous trees, because of the loss of the leaves and the sudden cessation of the sap flow in autumn and the sudden start of sap flow in spring.

Box 1.1

Studies on the relationship between xylem CO₂ concentration and CO₂ efflux

Only few studies have established the relationship between stem CO₂ efflux rates (E_{CO_2}) and the internal CO₂ concentration in the xylem ([CO₂] or [CO₂*]). Teskey and McGuire (2002) performed measurements of xylem [CO₂*] and E_{CO_2} *in situ* on mature trees of *Quercus alba* L., *Liriodendron tulipifera* L. and *Pinus taeda* L. trees. Xylem [CO₂*] was always positively correlated with stem E_{CO_2} . They conducted two types of experiments to determine the effect of xylem sap [CO₂*] on E_{CO_2} . CO₂ enriched water was pumped through detached branch segments in the laboratory and CO₂ enriched water was infused into the xylem of tree stems in the field. In both experiments, xylem [CO₂*] and E_{CO_2} significantly increased and a direct positive relationship was found between [CO₂*] and E_{CO_2} . Teskey and McGuire (2005) severed *Platanus occidentalis* L. and *Liquidambar styraciflua* L. saplings from their roots and placed them in cylinders filled with water with low, medium and high [CO₂*]. In response to the treatment, xylem [CO₂*] was low, medium or high, and xylem [CO₂*] explained 76-77% of the variation in stem E_{CO_2} . They also found that after drilling holes through the stem, E_{CO_2} increased 4- to 5-fold in *P. occidentalis* and 5- to 7-fold in *L. styraciflua*. They suggested that removing the barriers to CO₂ diffusion by drilling holes caused the large increase in E_{CO_2} . Maier and Clinton (2006) measured xylem [CO₂] and E_{CO_2} in young *Pinus taeda* L. tree stems during spring. They found that when the canopy foliage was removed, xylem [CO₂] increased, but there was no apparent change in E_{CO_2} . They assumed that during spring the cambium and phloem meristems likely respire at much higher rate than the xylem parenchyma and thus would be a major source of CO₂ in the stem (Figure 1.3 arrows 1a, 1b, 3a and 3b). Under these conditions the [CO₂] gradient would likely decrease from cambium to xylem, which rules out the xylem as a source of E_{CO_2} (Figure 1.3 arrow 1c is prevented).

1.3.2 Re-fixation of CO₂ by corticular and wood photosynthesis

At least five different locations of chloroplast containing tissues have been found in woody organs: (1) the rhytidome, (2) the ray parenchyma, (3) the tissues adjacent to the cork cambium, (4) the phloem and even (5) the wood and the region around the pith (Pfan­z and Aschan 2001) (Figure 1.5). Area-related chlorophyll contents of young twigs can be 50 – 70% and even more of the chlorophyll contents of adjacent leaves (Kharouk et al. 1995, Solhaug et al. 1995, Schmidt et al. 2000, Pfan­z et al. 2002). According to Kharouk et al. (1995), the bark of young *Populus tremuloides* Michx and *P. tremula* L. trees contains up to 42% of the total tree chlorophyll.

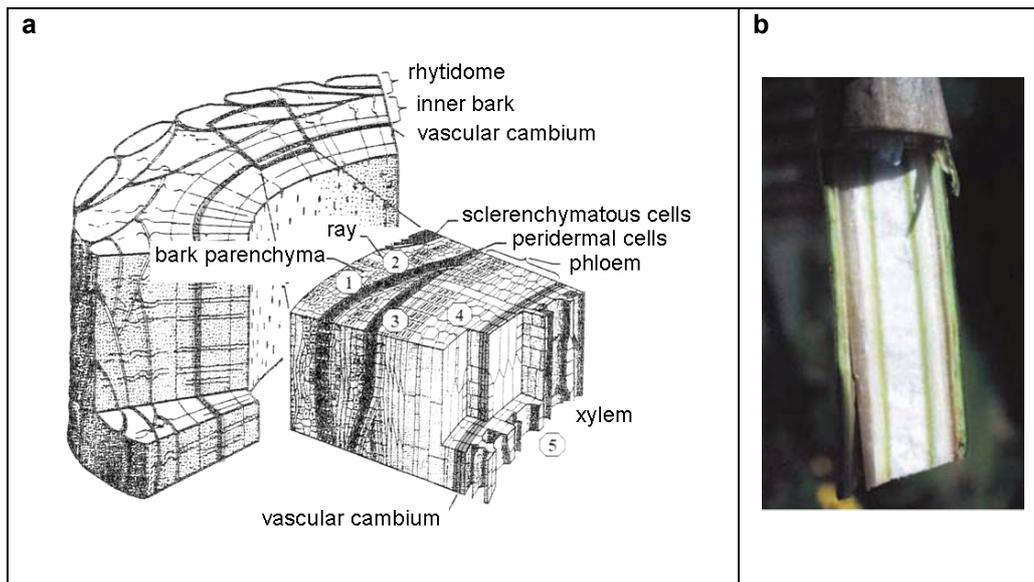


Figure 1.5: (a) Localization of chloroplast containing cells in a tree stem (adapted from Pfan­z and Aschan 2001). Numbers 1 – 5 refer to the text. (b) Cut twig of *Fraxinus excelsior* L., showing chlorophyll containing tissues in the bark layer and around the pith (adapted from Pfan­z et al. 2002).

Either artificial or natural illumination of tree stems typically decreases E_{CO_2} from the stem, indicating photosynthetic CO₂ assimilation by the chlorophyll containing tissues (Foote and Schaedle 1976a,b, Kharouk et al. 1995, Pfan­z 1999, Pfan­z et al. 2002). Particularly CO₂ produced by cells adjacent to these chloroplast containing cells is likely to be re-fixed (Figure 1.3, arrow 2). Internal recycling of carbon might be a highly effective way of reducing carbon loss during drought or winter periods (Pfan­z and Aschan 2001). Moreover, corticular and wood photosynthesis may be the only process able to partially enrich the O₂ concentrations of stems to avoid anoxic conditions developing in these organs (Pfan­z et al. 2002). Re-fixation has two advantages over leaf photosynthesis: (1) because no stomata are involved, corticular and wood photosynthesis is very little associated with water loss and (2) because [CO₂] in woody tissues is high, photorespiration is low (Cernusak and Marshall 2000). Stem internal CO₂ re-fixation, especially in young trees, may compensate for a large

fraction of the potential respiratory carbon loss, and in some cases even net photosynthetic CO₂ uptake has been observed (Table 1.2).

Table 1.2: Reported values of maximum stem internal CO₂ re-fixation rates. Re-fixation is estimated from CO₂ efflux in the dark ($E_{CO_2(d)}$) and under light ($E_{CO_2(l)}$): % re-fixation = $(E_{CO_2(d)} - E_{CO_2(l)})/E_{CO_2(d)} \times 100$. Values higher than 100% indicate net CO₂ uptake.

Species	Maximum re-fixation rate (%)	Age (years)	Reference
<i>Populus tremuloides</i> Michx	90 (winter) 92 (summer)	6 - 8	Foote and Schaedle (1976a)
<i>Acer rubrum</i> L.	31 (winter)	0 - 1	Coe and McLaughlin (1980)
<i>Cornus florida</i> L.	79 (winter)	0 - 1	Coe and McLaughlin (1980)
<i>Quercus alba</i> L.	19 (winter)	0 - 1	Coe and McLaughlin (1980)
<i>Liriodendron tulipifera</i> L.	23 (winter)	0 - 1	Coe and McLaughlin (1980)
<i>Fagus crenata</i> Blume	98	5	Han and Suzaki (1981)
<i>Pinus sylvestris</i> L.	45	2	Linder and Troeng (1981)
	5	12	
28 species	77		Comstock and Ehleringer (1988)
<i>Alnus glutinosa</i> (L.) Gaertn.	45	10	Steinborn et al. (1997)
<i>Guiera senegalensis</i> JF Gmel	76		Levy and Jarvis (1998)
<i>Pinus monticola</i> Dougl.	76	3 - 4	Cernusak and Marshall (2000)
<i>Populus tremuloides</i> Michx	80	0 - 1	Aschan et al. (2001)
<i>Populus tremula</i> L., <i>Fagus sylvatica</i> L.	90	0 - 1	Wittmann et al. (2001)
<i>Betula pendula</i> Roth	50	1.5	Matyssek et al. (2002)
<i>Fagus sylvatica</i> L.	110 (winter)	0 - 1	Damesin (2003)
<i>Prunus persica</i> L.	66	0 - 1	Alessio et al. (2005)
<i>Eucalyptus miniata</i> Cunn. Shauer	55	n.a.	Cernusak et al. (2006)
<i>Betula pendula</i> Roth	97	6	Wittmann et al. (2006)
<i>Alnus glutinosa</i> (L.) Gaertn.	122 (winter) 81 (summer)	0 - 1	Berveiller et al. (2007)
<i>Betula pendula</i> Roth	123 (winter) 69 (summer)	0 - 1	Berveiller et al. (2007)
<i>Fagus sylvatica</i> L.	114 (winter) 74 (summer)	0 - 1	Berveiller et al. (2007)
<i>Fraxinus excelsior</i> L.	126 (winter) 67 (summer)	0 - 1	Berveiller et al. (2007)
<i>Ginkgo biloba</i> L.	57 (winter) 67 (summer)	0 - 1	Berveiller et al. (2007)
<i>Picea abies</i> (L.) Karst.	50 (winter) 71 (summer)	0 - 1	Berveiller et al. (2007)
<i>Pinus sylvestris</i> L.	55 (winter) 69 (summer)	0 - 1	Berveiller et al. (2007)
<i>Quercus robur</i> L.	72 (winter) 69 (summer)	0 - 1	Berveiller et al. (2007)
<i>Tilia cordata</i> Mill.	75 (winter) 68 (summer)	0 - 1	Berveiller et al. (2007)

Several features are indispensable for a working CO₂ assimilation metabolism: (1) an effective chloroplast structure (Larcher et al. 1988), (2) photosynthesizing enzymes (Buns et al. 1993), (3) nutrients, (4) water, (5) photosynthetically active radiation (PAR) and (6) CO₂ (Pfan­z et al. 2002). An important limiting factor is PAR. For photosynthesis to occur sufficient PAR has to pass through the epidermal, peridermal and/or rhytidomal layers to reach the light-harvesting complexes of the chloroplasts. The amount of light transmitted in young trees varies between less than 10 to 50% of incident light, depending on species and age of the stem segment (Aschan and Pfan­z 2003). Light transmission usually decreases with stem age. The older the bark, the higher the absorption and/or reflection of the penetrating light and the lower the transmitted portion (Kharouk et al. 1995, Pfan­z and Aschan 2001). In contrast with leaf photosynthesis, CO₂ is not a limiting factor for corticular photosynthesis. Due to the low permeability to gaseous diffusion of peridermal and rhytidomal layers, CO₂ accumulates in the stem, reaching high internal concentrations (Table 1.1).

Box 1.2

Studies on the relationship between internal CO₂ concentration and corticular photosynthesis

Cernusak and Marshall (2000) found that mean bark [CO₂] in illuminated (1000 μmol PAR m⁻² s⁻¹) branches of *Pinus monticola* Dougl. ex D. Don was 869 ppm, while it was 1726 ppm in the dark. Wittmann et al. (2006) found that the mean bark [CO₂] in *Betula pendula* Roth branches was 618 ppm and 1548 ppm in the light (600 μmol PAR m⁻² s⁻¹) and dark, respectively. These results indicate that a substantial amount of the CO₂ present in the bark is re-fixed by chloroplast containing tissues of the stem. We are unaware of any studies about the effect of corticular photosynthesis on xylem or phloem [CO₂]. Nevertheless, since chlorophyll containing cells are also located in this tissue (Figure 1.5), it is very probable that also xylem and phloem [CO₂] are affected by photosynthesis.

1.3.3 Export of dissolved CO₂ with the transpiration stream

Already in the beginning of the 20th century it has been speculated by Boysen-Jensen (1933) and Johansson (1933) that transpiration may significantly affect the stem internal [CO₂]. A great part of the respiring tissues in tree stems is either perfused by the transpiration stream (xylem parenchyma) or is immediately adjacent to the transpiration stream (vascular cambium). According to Henry's law the total amount of CO₂ dissolved in the xylem sap ([CO₂*]) depends on temperature, pH and the CO₂ concentration in the gaseous phase surrounding the sap ([CO₂]). Due to the respiration processes in the stem and the high resistances for radial CO₂ diffusion, [CO₂] in the stem is generally very high (Table 1.1) and a part of the respired CO₂ is dissolved in the sap (Figure 1.3, arrows 3a, 3b and 3c). Particularly CO₂ produced by

xylem parenchyma (arrow 3c) is likely to be dissolved in the sap since it is directly surrounded by the conducting sap. During the day, this sap is subjected to a (negative) pressure gradient created by the transpiring leaves and the CO₂ dissolved in the xylem sap is transported via bulk flow.

The fate of xylem-transported CO₂ is unknown. Some transported CO₂ may be released from the sap at branch or twig level and evolve to the atmosphere; some transported CO₂ may be re-fixed by corticular, wood or leaf photosynthesis. Teskey and McGuire (2002) reasoned that much of the xylem-transported CO₂ is likely to be re-fixed by leaf photosynthesis. This seems indeed very probable since [CO₂] in leaves is very low and forms a limiting factor for leaf photosynthesis. For example, Rey and Jarvis (1998) reported that the [CO₂] in the mesophyll of *Betula pendula* Roth leaves was only 0.022%. Zelawski et al. (1970) added water with ¹⁴CO₂ dissolved in it to the stem transpiration stream of *Pinus elliotii* Engelm. seedlings and found that large amounts of ¹⁴CO₂ were fixed in needles and stems. Also a large amount of internal CO₂ evolved to the atmosphere, but it diffused primarily out of the stem, not the needles. Stringer and Kimmerer (1993) examined the re-fixation of xylem sap CO₂ in *Populus deltoides* Bartr. Ex Marsh. leaves. Excised leaves were allowed to transpire a 1 mM [¹⁴C]NaHCO₃ solution. They found that 99.6% of the label was fixed in the veins under illumination. Hibberd and Quick (2002) found that when [¹⁴C]NaHCO₃ was supplied to tobacco roots, ¹⁴C accumulated in insoluble material, most notably starch, in cells associated with the vascular system.

Box 1.3

Studies on the relationship between xylem CO₂ concentration and sap flow

As far as we know, the relationship between xylem CO₂ concentrations and sap flow rate (f_s) has only been investigated by two research groups. Teskey and McGuire (2002) performed measurements of [CO₂*] and f_s on mature trees of *Quercus alba* L., *Liriodendron tulipifera* L. and *Pinus taeda* L. trees. They found that diurnal patterns of xylem [CO₂*] were negatively correlated with diurnal changes in f_s . For example, decreases in f_s caused by passing clouds corresponded with increases in [CO₂*]. They concluded that sap flow had a substantial influence on xylem sap [CO₂*]. Maier and Clinton (2006) measured xylem [CO₂] and f_s in young *Pinus taeda* L. tree stems during spring while most of the canopy foliage was removed. In uncut trees, xylem [CO₂] generally decreased during the day when f_s was high and increased at night when f_s was low. After the removal of most of the canopy and a subsequent decrease of f_s to near zero, [CO₂] exhibited a substantial increase, suggesting that f_s had a strong influence on xylem [CO₂].

1.4 Quantifying stem respiration

Two basic methods have been applied to measure woody tissue respiration (R_S), although neither is totally satisfactory. The first method (e.g., Yoda et al. 1965, Kinerson 1975, Hagihara and Hozumi 1981, Zabuga and Zabuga 1990, Pruyn et al. 2002a,b, Bowman et al. 2005) is to bring cut sections of stems and branches to the laboratory, enclose them in a cuvette and measure their CO₂ efflux (E_{CO_2}) or O₂ consumption under controlled conditions. The second method is to attach permanent cuvettes to living trees, and measure E_{CO_2} continuously over the course of one or more growing seasons. In both methods, it is assumed that all the CO₂ generated from the living stem tissues enclosed within the cuvette diffuses from the tissue interior to the surface, so that E_{CO_2} reflects R_S of the stem tissue.

However, it has been questioned by several researchers whether this measured E_{CO_2} reflects the actual R_S of the tissue. The reasons for this concern have implicitly been mentioned throughout this literature review.

In paragraph 1.3.1 it has been explained that woody tissue E_{CO_2} depends on the resistance to radial CO₂ diffusion of the wood, cambium and bark layers. CO₂ concentrations in the gas phase of the xylem can surmount the atmospheric concentration by up to three orders of magnitude (Table 1.1), indicating that the overall diffusion resistance is large. It has been claimed that measured stem E_{CO_2} originates physico-chemically from a strong diffusion gradient, creating a continuous, largely temperature-independent CO₂ efflux, rather than from actual R_S of living tissues (Gansert and Burgdorf 2005). In that case, there is a discrepancy between the actual R_S and E_{CO_2} , which implies that techniques based on measurements of E_{CO_2} as described above are not adequate for measuring R_S .

In paragraph 1.3.3 the influence of f_s on stem internal [CO₂] has been discussed. Respired CO₂ at stem level can be dissolved in the xylem sap and may be carried upward with the transpiration stream instead of diffusing radially through the bark (e.g., Boysen-Jensen 1933, Johannson 1933). Export of dissolved CO₂ by the transpiration stream results in a negative relationship between f_s and stem E_{CO_2} , which has frequently been observed. Negisi (1979) measured E_{CO_2} from detached stem segments of young *Pinus densiflora* Sieb. et Zucc. trees through which the flow rate of water was controlled using a pump. When f_s was gradually increased, E_{CO_2} gradually decreased. Martin et al. (1994) varied transpiration rates of *Pinus taeda* L. seedlings by changing the absolute humidity deficit, while all other environmental variables were maintained constant. Stem E_{CO_2} was 6.7% lower during periods of high transpiration than during periods of low transpiration. Kaipainen et al. (1998) observed low E_{CO_2} of *Pinus sylvestris* L. trunks and branches during midday in summer, when f_s was high. McGuire and Teskey (2004) observed opposite trends in f_s and E_{CO_2} in a *Fagus grandifolia* Ehrh., *Platanus occidentalis* L. and *Liquidambar*

styraciflua L. tree. Gansert and Burgdorf (2005) studied the effect of xylem f_s on E_{CO_2} in stems of mature *Betula pendula* Roth trees and found a negative correlation, particularly during July and August. It was hypothesized by the authors that a part of the respired CO_2 is transported away from the stem segment enclosed by the cuvette, so that E_{CO_2} underestimates the actual R_S . When applying the first technique (i.e., enclosing cut woody tissue sections in a cuvette and measuring E_{CO_2}), the stem or branch is detached from the transpiration stream, so that the transport of respired CO_2 is not an issue. Contrarily, when using the second method (i.e., measuring E_{CO_2} from intact stems and branches enclosed in a cuvette), CO_2 transport might influence E_{CO_2} , so that measured E_{CO_2} underestimates R_S during the daytime.

The transpiration stream may also be a source of CO_2 when CO_2 originating from root tissue or soil microbial respiration is dissolved in the xylem sap, carried upward and be released at stem level (Levy et al. 1999, Teskey and McGuire 2007). Import of CO_2 results in a positive relationship between f_s and E_{CO_2} , which has been observed by Levy et al. (1999). They examined f_s and stem E_{CO_2} of the shrub *Combretum micranthum* G. Don. and found that, after removing the temperature effect on E_{CO_2} using Equation 1.2, the two processes were positively related. Therefore they assumed that the water taken up by the shrub was highly CO_2 enriched and that CO_2 was released from the water at stem level, resulting in a higher stem E_{CO_2} . In that case, the transpiration stream is a CO_2 source, and measured E_{CO_2} from non-detached segments enclosed by a cuvette is the combined result of the actual R_S and the release of CO_2 from the transpiration stream. In other words, measured E_{CO_2} overestimates actual R_S . Again, the first method is not faced with this problem, since the stem tissue is detached from the transpiration stream.

In contrast with the previous studies, Wittmann et al. (2006) measured E_{CO_2} of *Betula pendula* Roth branches before and after excision and found no significant differences, indicating that f_s had no influence on branch E_{CO_2} . They concluded from these results that branch E_{CO_2} was mainly generated from the cambium and phloem cells, whereas the contribution of xylem CO_2 to E_{CO_2} was negligible. Maier and Clinton (2006) found that removal of a part or the entire crown and, hence, decreasing f_s , did not alter stem E_{CO_2} of *Pinus taeda* L. trees. They concluded that R_S of the external stem tissues had likely the largest influence on stem E_{CO_2} because of its high metabolic activity and its proximity to the stem surface. Following the reasoning of these authors, measured E_{CO_2} is only a reflection of R_S of the external living stem tissues (cambium and phloem) and not of R_S of the xylem living tissues. In that case, both techniques for estimating woody tissue R_S underestimate the actual R_S because R_S of the xylem living tissues is not accounted for. Maier and Clinton (2006) added in their discussion that measurements of E_{CO_2} on intact stem or branch segments probably even underestimate R_S of the external woody tissues as some of their respired CO_2 is expended into the xylem, where it might be transported

with the transpiration stream. Again, the first technique does not cope with this problem, since the segments are detached from the transpiration stream.

In conclusion, it appears that the first method would be a better method to quantify actual woody tissue R_S . However, this method has to deal with some major disadvantages: (1) it does not allow repeated measurements on a single branch or stem, (2) it may stimulate traumatic or wound respiration, (3) water loss from the tissue is unavoidable, which could result in a decrease of R_S (paragraph 1.2.3.2), (4) it detaches the stem or branch from its normal supply of water, nutrients and plant growth substances, which could affect R_S in ways that are not easily predictable and (5) it causes rapid diffusion of CO₂ because diffusion barriers are removed upon excision (Sprugel 1990, Teskey and McGuire 2005). Therefore, the second technique, measuring E_{CO_2} from intact stem or branch segments, is more commonly applied in woody tissue respiration studies.

McGuire and Teskey (2004) have proposed a mass balance approach for estimating R_S of an intact (non-detached) stem segment based on measurements of E_{CO_2} of the stem segment, f_s and xylem [CO₂] above and below the segment. The mass balance accounts for both the external (i.e., E_{CO_2}) and internal fluxes of CO₂. The internal fluxes comprise the import (I_T) and export (E_T) of dissolved CO₂ into and out of the stem segment by flowing sap and the transient changes in the storage of CO₂ within the stem (ΔS) (Figure 1.6).

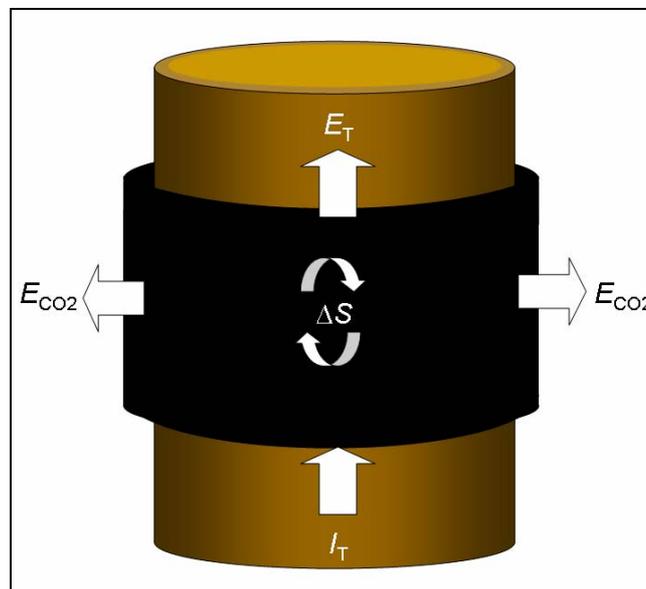


Figure 1.6: Schematic of the internal and external fluxes of CO₂ of a tree stem segment enclosed in a cuvette (black cylinder). E_{CO_2} = CO₂ efflux to the atmosphere, E_T = export of dissolved CO₂ out of the stem segment, I_T = import of dissolved CO₂ into the stem segment, ΔS = change in internal CO₂ concentration over time (adapted from McGuire and Teskey 2004).

The transport flux F_T (i.e., $E_T - I_T$) ($\mu\text{mol CO}_2 \text{ m}^{-3} \text{ s}^{-1}$) is calculated as:

$$F_T = (f_s/V) \times \Delta[\text{CO}_2^*] \quad (1.4)$$

where f_s is the sap flow rate through the segment (l s^{-1}), V the sapwood volume of stem segment (m^3) and $\Delta[\text{CO}_2^*]$ the difference in DIC concentration in the xylem above and below the cuvette ($\mu\text{mol l}^{-1}$).

The storage flux ($\mu\text{mol CO}_2 \text{ m}^{-3} \text{ s}^{-1}$) is calculated as:

$$\Delta S = (\overline{[\text{CO}_2^*]}_{t1} - \overline{[\text{CO}_2^*]}_{t0}) \times w/t \quad (1.5)$$

where $\overline{[\text{CO}_2^*]}_{t1}$ and $\overline{[\text{CO}_2^*]}_{t0}$ are means of upper and lower $[\text{CO}_2^*]$ in the stem segment at time $t1$ and $t0$, respectively, w is the specific water content of the stem segment (l m^{-3}) and t is the time interval ($t1-t0$).

McGuire and Teskey (2004) found that over a 24-h period E_{CO_2} represented as much as 83% (in *Liquidambar styraciflua* L.) and as little as 45% (in *Platanus occidentalis* L.) of R_S . During the daytime, E_{CO_2} in *P. occidentalis* represented only 23% of R_S and F_T accounted for 71% of R_S . Their study indicated that efflux based estimates of stem respiration may introduce large errors, particularly during the daytime, when sap flow is high. They recommended adopting the terminology 'CO₂ efflux' rather than 'respiration' to depict measurements of CO₂ release from woody tissues to the atmosphere.

1.5 Conclusions

This chapter attempted to combine the today's available literature on the sources and sinks of stem internal CO₂ and the main factors which they depend on. The major source of stem internal CO₂ is the local respiration of the living stem cells. Cambium and phloem have been found to be the most metabolically active stem tissues. The rate of metabolic activity of the living cells depends on several factors, such as temperature and growth rate. Also nutrient, water, O₂ and carbohydrate availability have been put forward as variables affecting stem metabolism, but clear and indisputable evidence for their influence on stem respiration rates has not been provided so far. Besides respiration, also import of CO₂ by the transpiration stream might be an important source of stem internal CO₂. CO₂ in the xylem stream originates from soil water and/or root respiration. Our understanding of the influence of soil and root CO₂ on the stem internal CO₂ concentration is still incomplete. Further research needs to be conducted in order to reveal the contribution of this component to the carbon balance of tree stems.

An important sink of stem internal CO₂ is radial diffusion to the atmosphere. However, the large difference in CO₂ concentration between the stem interior and the atmosphere indicates that radial CO₂ diffusion from the stem to the atmosphere is

severely restricted by diffusion barriers. Although their impact on CO₂ diffusion rates may be substantial, scientists studying woody tissue respiration have rarely taken these barriers into consideration. Few researchers have attempted to quantify the diffusion resistance of bark, cambium and xylem. Diffusion occurs both in the gaseous and aqueous phase of the stem, but the latter occurs 10⁴ times slower than the former. This implies that the ratio gas/water content of the stem may have an important influence on the diffusion rate. Another sink of stem internal CO₂ is cortical and wood photosynthesis. Stem internal re-fixation may compensate for a large fraction of potential respiratory carbon loss and in some cases even net photosynthetic CO₂ uptake may occur. Cortical or wood photosynthetic rates depend on several factors, such as the availability of an effective chloroplast structure, photosynthesizing enzymes, nutrients, water, photosynthetically active radiation and CO₂. Particularly radiation may be a limiting factor due to the low light transmittance of the bark. Export of CO₂ by the xylem stream is a third sink of stem internal CO₂, which represents a potentially large and poorly understood carbon flux in trees. The transport of dissolved CO₂ with the transpiration stream depends on the sap flow rate and the solubility of CO₂ in xylem sap. The latter is determined by the temperature and pH of the sap and by the CO₂ concentration in the gaseous phase surrounding the sap. The fate of transported stem respiratory CO₂ is unknown, but it is likely that it is delivered to the leaves, where it is re-fixed in photosynthesis.

As long as woody tissue respiration has been studied, it has been pointed out that quantifying the actual stem respiration rates is faced with several difficulties, which have not yet been solved. The majority of the scientists now realizes that woody tissue respiration can not simply be approximated by the stem CO₂ efflux rate. Recently a mass balance approach for the calculation of actual stem respiration rate has been developed (McGuire and Teskey 2004), accounting for CO₂ effluxes as well as CO₂ storage and internal CO₂ fluxes in the xylem. However, future work needs to expand on this model. For example, it still needs to be proved that it is capable of predicting actual respiration rates. Furthermore, it should be tested whether this balance is valid for different tree species, soil types and climatic conditions.

Chapter 2

Measuring variables related to stem respiration

2.1 Introduction

As mentioned in Chapter 1, studying stem respiration (R_S) nowadays comprises more than measuring the CO_2 efflux rate (E_{CO_2}) from a stem segment. If we want to refine estimates of R_S , several other variables have to be taken into consideration.

An interesting variable is the xylem CO_2 concentration ($[\text{CO}_2]$). $[\text{CO}_2]$ in the gas phase of the xylem in tree stems has been measured since the early 20th century (Table 1.1), but only recently techniques have been developed for continuous *in situ* measurements of xylem $[\text{CO}_2]$ (McGuire and Teskey 2002, Teskey and McGuire 2007). The focus of this chapter will be on the applied techniques for measuring xylem $[\text{CO}_2]$, because these techniques may be less known by the majority of the readers. We begin by briefly reviewing xylem $[\text{CO}_2]$ measurement techniques used in the past. The sections that follow discuss the recently developed *in situ* techniques more comprehensively.

Besides $[\text{CO}_2]$, also other variables are involved in stem respiration studies. In paragraph 1.3.3 it has been discussed that the transpiration stream is capable of importing CO_2 into a stem segment or exporting CO_2 out of a stem segment. To account for this flux, it is essential to measure the sap flow rate (f_s) through the stem. In paragraph 1.2.3.2, it has been highlighted that stem R_S can be affected by several factors, such as growth rate, temperature, nutrient status and water, O_2 and carbohydrate availability. Although all of potential great importance, this thesis restricts to the effects of growth rate, temperature and water status. Measurements of E_{CO_2} , f_s , growth rate and water status are common in tree physiological studies. We therefore restrict the discussion to the experienced virtues and shortcomings of the different techniques and their applicability in stem respiration studies.

2.2 CO_2 efflux measurements

To measure stem CO_2 efflux rates (E_{CO_2}) in this thesis, intact (non-detached) stem segments were enclosed in fan-stirred cuvettes (Figure 2.1), which were covered with aluminium foil in order to prevent cortical and wood photosynthesis. Cuvettes

were connected with Teflon tubing to an infrared gas analyzer (IRGA). Heteratomic gas molecules such as CO₂ typically absorb radiation at specific infrared (IR) wavebands, each gas having a characteristic absorption spectrum. CO₂ has three large absorption bands in the IR region at about 2.7, 4.3 and 15 μm. The only heteratomic gas normally present in air with an absorption spectrum overlapping that of CO₂ is water vapour (both molecules absorb IR in the 2.7 μm wavelength region) (Janáč et al. 1971). Since water vapour is usually present in much higher concentrations than CO₂, this interference may present a significant problem. Therefore, the air leaving the stem cuvette was dried before being analyzed by the IRGA. Two methods were applied in this thesis: (1) a gas cooler (Model CG/G 73-4, Hartmann and Braun, Frankfurt, Germany) operating at 4 °C, and (2) a column of the chemical desiccant Mg(ClO₄)₂ (magnesium perchlorate) (Figure 2.2). Gas analysis was performed in open configuration, meaning that air from outside the system was pumped at a constant flow rate through the cuvette enclosing the stem segment. The supplied air was either compressed air of known near-ambient [CO₂] or ambient air, which was pumped into the cuvette via a 50 l buffer tank in order to minimize short-term fluctuations in CO₂ and H₂O concentrations (Figure 2.2).

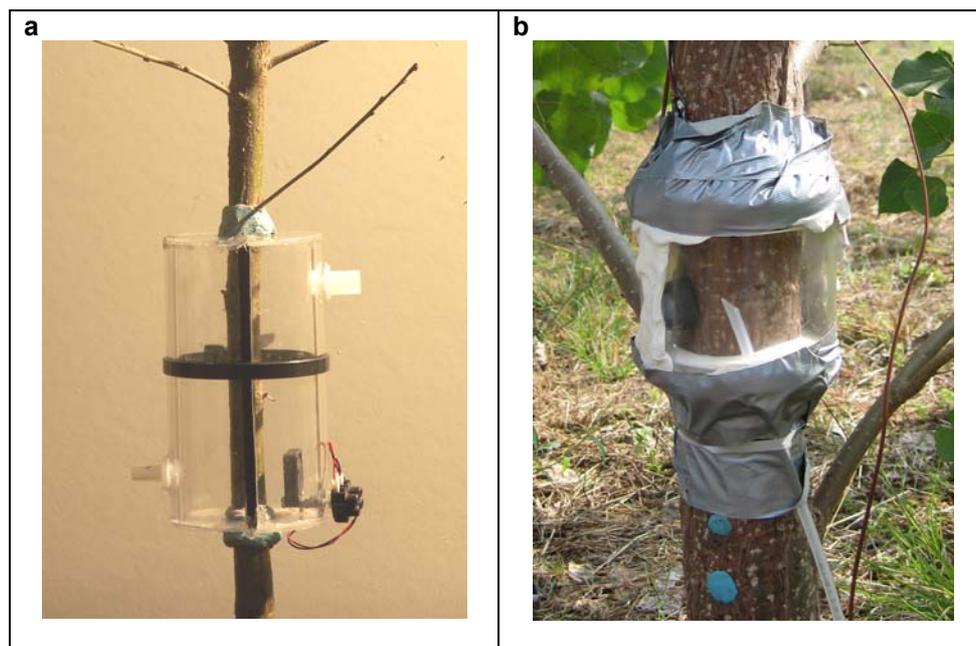


Figure 2.1: Fan-stirred cuvettes for continuous measurement of CO₂ efflux from a stem segment of (a) a young oak tree in the growth chamber and (b) an eastern cottonwood tree in the field. To avoid overheating from direct light and CO₂ re-fixation by photosynthesis, light was excluded by covering the cuvettes with several layers of aluminium foil. The cuvettes are sealed to the tree surface with flexible putty adhesive .

When ambient air was used, the IRGA was configured in differential mode. In that case, the IRGA measured both [CO₂] of the reference air and measurement air. The reference air was the air pumped through a reference cuvette, with the same dimensions of the stem cuvette, but containing no stem segment (Figure 2.2a). When

compressed air of known $[CO_2]$ was used, the IRGA was configured in absolute mode: only $[CO_2]$ of the measurement air was measured and $[CO_2]$ of the reference air was equal to the known $[CO_2]$ of the compressed air. The stem CO_2 efflux rate (E_{CO_2}) ($\mu\text{mol m}^{-2} \text{s}^{-1}$) was calculated as:

$$E_{CO_2} = (f_a/A) \times \Delta[CO_2] \quad (2.1)$$

where f_a is the rate of air flowing (mol s^{-1}) through the cuvette, A is the surface area of the stem segment (m^2) and $\Delta[CO_2]$ is the difference in $[CO_2]$ of measurement air and reference air ($\mu\text{mol mol}^{-1}$).

When the stem segment was larger, E_{CO_2} was expressed per unit sapwood volume ($\mu\text{mol m}^{-3} \text{s}^{-1}$):

$$E_{CO_2} = (f_a/V) \times \Delta[CO_2] \quad (2.2)$$

where V is the sapwood volume of the segment (m^3).

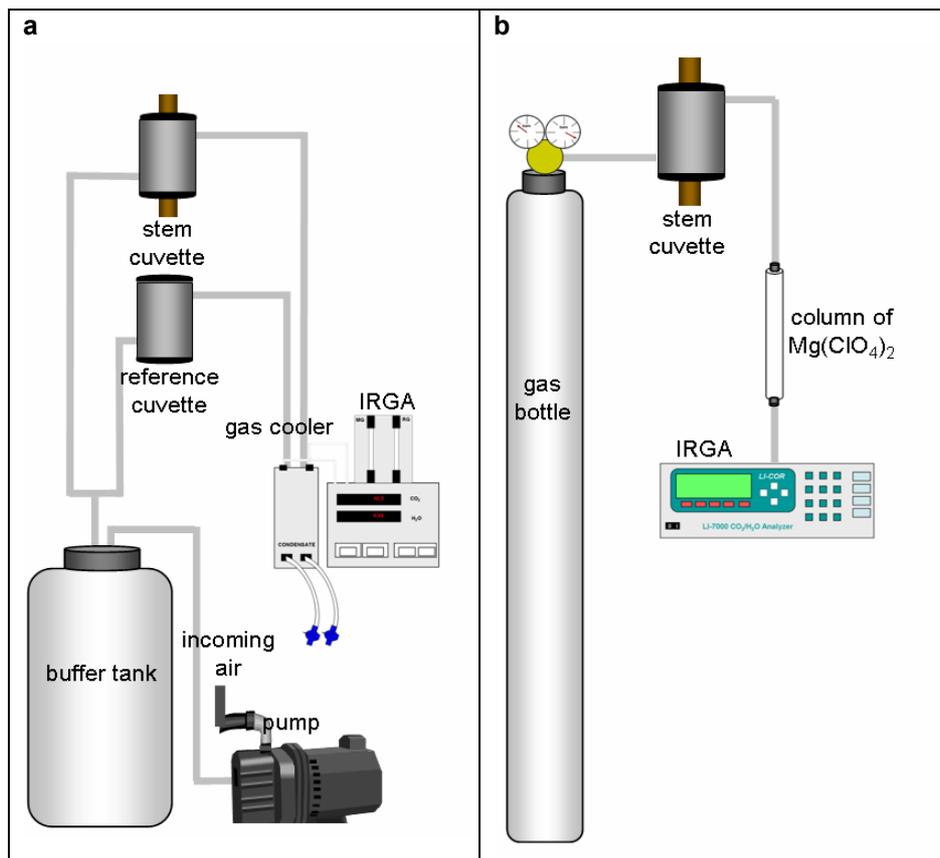


Figure 2.2: Schematic of the different setups for CO_2 efflux measurements: (a) ambient air is pumped into a stem cuvette and reference cuvette via a buffer tank and the air leaving the cuvettes is dried with a gas cooler before being analyzed by an infrared gas analyzer (IRGA) configured in differential mode; (b) compressed air of known CO_2 concentration is pumped into a stem cuvette and the air leaving the cuvette is dried over a column of magnesium perchlorate before being analyzed by an IRGA configured in absolute mode.

CO₂ efflux measurements achieved with the 'compressed air method' were generally more stable than with the 'ambient air method', but also the compressed air method was faced with shortcomings. The use of air bottles and magnesium perchlorate raised the cost of the measurements considerably compared to the ambient air method and was therefore less suitable for long-term measurements. Moreover, the setup required frequent inspection as the air bottles ran empty rather fast.

2.3 Measurements of xylem CO₂ concentration

The first methods for measuring stem internal [CO₂] were based on the extraction of gas from a hole bored into the stem or from a cuvette placed onto the stem surface. After the extraction, the CO₂ concentration of the gas was determined by chemical absorption using NaOH or KOH (MacDougal 1927, MacDougal and Working 1933, Chase 1934, Jensen 1967), by gas chromatography – mass spectrometry (Eklund 1990, 1993) or by infrared gas analysis (IRGA) (Hari et al. 1991, Levy et al. 1999, Ceschia 2001). A disadvantage of drilling holes into the stem is that wound respiration may influence the results. However, by using a cuvette onto the stem surface, underestimation of [CO₂] may occur because of diffusion through the bark around the cuvette seal and because of incomplete equilibration of the internal and external [CO₂] due to the large resistance for CO₂ diffusion of cambium and bark. A general difficulty with the extraction of gases from a hole or from cuvette is that a negative pressure in the hole or cuvette is created, which increases the risk for leaks. Extraction of gases from a hole or cuvette can only be performed at larger time intervals, since the gas in the hole or cuvette needs to re-equilibrate with the xylem gaseous phase after each sampling. Diurnal variations in xylem [CO₂] can not be monitored using these methods. Recently, new techniques for continuous *in situ* monitoring of xylem [CO₂] have been developed. These techniques make use of CO₂ sensors which are mounted into holes drilled in the stem. The applied sensors are the CO₂ microelectrode (Model MI-720, Microelectrodes Inc., Bedford, NH, USA) and the solid state non-dispersive infrared (NDIR) CO₂ sensor (Model GMM 221, Vaisala, Helsinki, Finland). These sensors measure CO₂ concentration in the gas phase of the xylem, which is in equilibrium with the concentration of all products of CO₂ dissolved in the aqueous phase of the xylem.

To monitor xylem [CO₂], three different methods were applied in this thesis: (1) gas extraction from holes in the stem in combination with gas chromatography, (2) *in situ* monitoring of [CO₂] in holes in the stem with CO₂ microelectrodes and (3) *in situ* monitoring of [CO₂] in holes in the stem with NDIR sensors. The following paragraph gives a technical description of the methods and sensors. Some typical results of the different techniques are presented, followed by a discussion of their advantages and disadvantages and their applicability in different situations. Finally, the method to convert CO₂ concentration in the gas phase of the xylem ([CO₂]) to the concentration

of all products of CO₂ dissolved in the aqueous phase of the xylem ([CO₂*]) is explained.

2.3.1 Technical description of [CO₂] measuring techniques

2.3.1.1 Gas extraction and gas chromatography

This technique was applied on a young beech (*Fagus sylvatica* L.) and oak (*Quercus robur* L.) tree in a growth chamber under controlled standard conditions: 12/12-h photoperiod, 470 μmol m⁻² s⁻¹ photosynthetically active radiation (PAR) at the top of the trees, constant air temperature (21 ± 2 °C), two-weekly watering and monthly fertilizing. Six-mm diameter holes were drilled 7 mm deep into the xylem at 4 different heights: 0.2, 0.35, 0.7 and 0.85 m. A Teflon tube (5 mm inner diameter and 25 mm length) was tightly fitted into each hole and the tube was sealed to the tree using flexible putty adhesive (Terostat IX, Henkel GmbH, Heidelberg, Germany). The seal prevented rapid escape of CO₂ from the xylem towards the atmosphere, caused by the sudden reduction of the barriers to CO₂ diffusion (i.e., the cambial and peridermal layers) (Teskey and McGuire 2005). A gas-tight rubber septum (Model Z10, 071-4, Sigma-Aldrich, Bornem, Belgium) was inserted into the end of each tube. The gas volume in the sealed tube was approximately 0.5 ml. 100 μl samples of the gas inside the tube were taken with a gas-tight syringe (Model 81230, Hamilton, Bonaduz, Switzerland). The gas was injected into a gas chromatograph (GC) (Model 14B, Shimadzu, Duisburg, Germany) with an electron capture detector. The GC was supplied with a Porapack Q preparatory column (1 m × 2 mm) and a Porapack Q separating column (2 m × 2 mm). Nitrogen was used as a carrier gas. Temperatures in the injector, oven and detector were 100, 35 and 250 °C, respectively.

For the calibration of the GC, five sample volumes of compressed gas containing 1% CO₂ were injected into the instrument. The amount of CO₂ in the sample volumes was calculated using the ideal gas law equation:

$$p \times V = n \times R_u \times T \quad (2.3)$$

with p the atmospheric pressure (Pa), V the sample volume (m³), n the amount of gas (mol), R_u the universal gas constant (8.314 J K⁻¹ mol⁻¹) and T the absolute temperature (K). The response of the GC to the amount of CO₂ can be described by the following equation:

$$[\text{CO}_2] = \frac{a \times \ln(1 - b \times \text{peak})}{V} \quad (2.4)$$

where peak is the output of the GC, V is the sample volume and a and b are regression coefficients.

2.3.1.2 CO₂ microelectrode

This technique was applied in growth chambers on young beech, oak and eastern cottonwood (*Populus deltoides* Bartr. ex Marsh.) stems. Holes of 4 mm diameter and 7 or 10 mm length (depending on the stem diameter) were drilled into the xylem and a Teflon or brass tube (3.5 mm inner diameter and 30 mm length) was tightly fitted into each hole. The tube was sealed to the tree using flexible putty adhesive (Terostat IX, Henkel, Heidelberg, Germany). The microelectrode was inserted in the tube and putty adhesive or Parafilm M film (American National Can, Menasha, WI, USA) was used to seal the junction between the tube and the sensor (Figure 2.3a).

The CO₂ microelectrode consists of a glass pH electrode and a reference Ag-AgCl electrode, which are enclosed in a replaceable plastic housing with a gas-permeable Teflon membrane tip (Figure 2.3b). The glass pH electrode consists of a glass membrane with a measurement Ag-AgCl electrode inside and filled with a NaCl solution. The plastic housing is filled with a NaHCO₃/NaCl electrolyte solution. A thin film of the solution separates the tip of the pH electrode from the gas-permeable membrane. CO₂ from the headspace of the hole diffuses through the membrane until the CO₂ partial pressure ($p\text{CO}_2$) of the hole and the electrolyte film are equal. The CO₂ reacts with the water of the electrolyte solution and H₃O⁺ is formed, which results in a pH change of the solution. The pH difference between the solutions inside and outside the glass membrane creates a potential difference between the measurement and reference Ag-AgCl electrodes, in proportion to the pH difference.

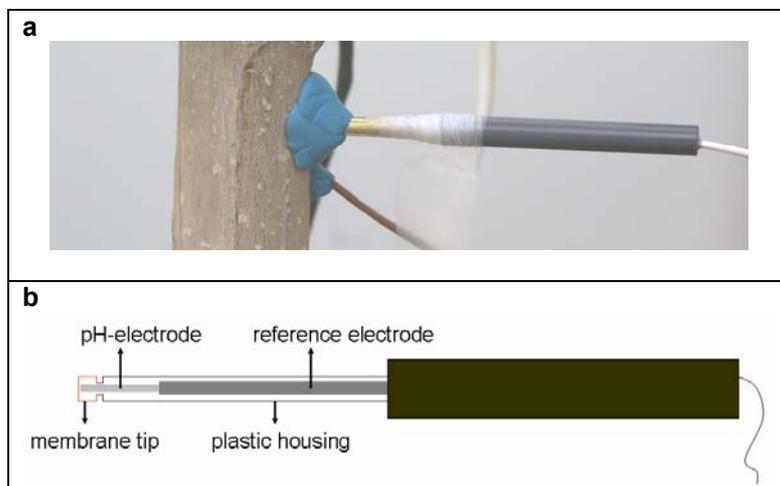


Figure 2.3: (a) Mounting of the CO₂ microelectrode in the stem of an eastern cottonwood tree. Flexible putty adhesive at the junction of the stem and tube and Parafilm M film at the junction of the tube and sensor provide an airtight seal. (b) Schematic of the CO₂ microelectrode.

For the calibration, gas with known [CO₂] was bubbled through 0.5 l water in a 1 l beaker, sealed with a rubber stopper (Figure 2.4a). Microelectrodes were inserted in holes in the stopper and suspended above the water. The beaker was placed into the water bath of a thermo-cryostat (UKT 2, Edmund Bühler/Otto GmbH, Germany) to regulate temperature. Because of the temperature-dependent response of the

microelectrodes, calibrations were conducted at several temperatures (usually 8 steps, comprising a temperature range between 10 and 30 °C). The calibration was performed with gas at 0, 2.5, 5 and 10% CO₂. The potential difference (V) was recorded with a data logger (HP 34970A, Hewlett Packard, Palo Alto, CA, USA) at 10-s intervals.

The microelectrode response to [CO₂] and temperature can be described by the following equation:

$$[\text{CO}_2] = (a \times T + b) \times e^{c \times U} \quad (2.5)$$

where [CO₂] is the CO₂ concentration of the gas (%), T the temperature (°C), U the potential difference (mV) and a , b and c regression coefficients. Figure 2.4b displays an example of a calibration graph.

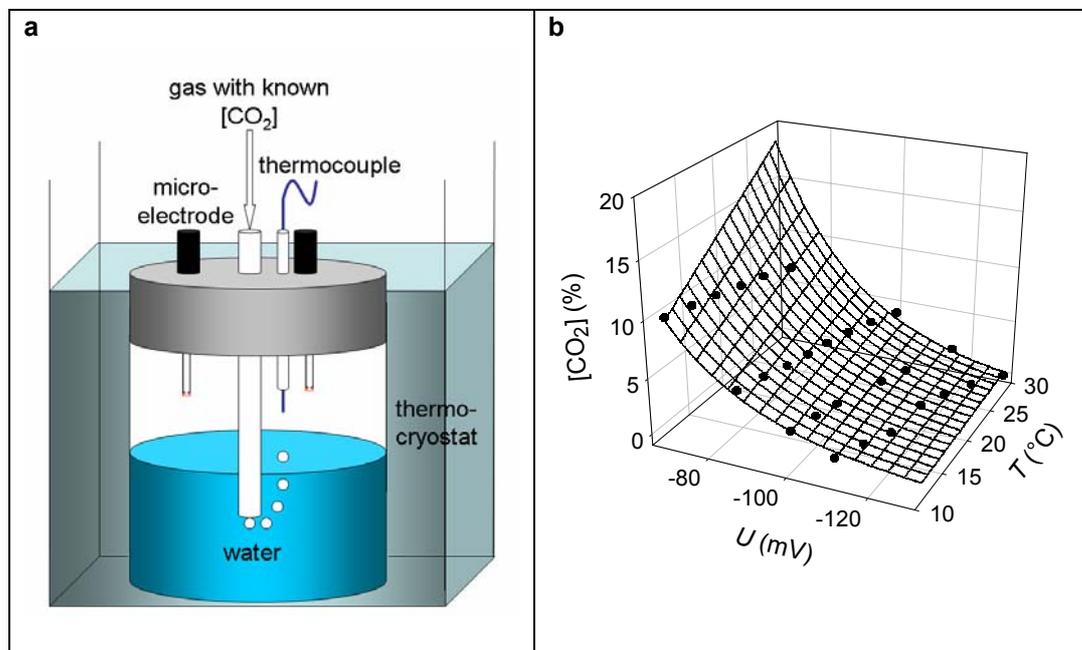


Figure 2.4: (a) Assembly for the calibration of the CO₂ microelectrodes. (b) Typical calibration curve for a CO₂ microelectrode. Equation 2.5 coefficients are $a = 10.7$, $b = 68.0$ and $c = 0.0437$. $R^2 = 0.99$.

2.3.1.3 Solid state non-dispersive infrared sensor

The NDIR sensor (Model GMM 221, Vaisala, Helsinki, Finland) could not be applied on small trees in the growth chamber because of its large dimensions (18.5 mm diameter and a 35-mm long sensor head). The technique was tested on eastern cottonwood in a field near Whitehall Forest, an experimental forest of the University of Georgia near Athens, Georgia, USA. Nineteen-mm diameter holes were drilled 50 mm deep into the stem at two heights: 0.45 and 0.64 m. The sensors were inserted in the holes and flexible putty adhesive (Qubitac, Qubit Systems, Kingston, Ontario,

Canada) was used to provide a gas-tight seal at the junction of the sensors and stem (Figure 2.6a).

The NDIR sensor consists of a broadband IR source at the end of a measurement chamber, which emits light into the chamber, where CO₂ molecules absorb a part of the light at their characteristic wavelength. The sensor contains a small electrically controlled Fabry-Perot interference filter (Figure 2.6b). First, the filter is tuned so that its pass band coincides with the absorption wavelength of CO₂. A detector measures the strength of the signal that passes through the filter. Second, the pass band of the filter is shifted to a wavelength where no absorption occurs. This provides the reference signal. The ratio of these two signals indicates the degree of light absorption by the CO₂ molecules and thus the CO₂ concentration. The housing of the sensor contains small holes and a membrane through which CO₂ diffuses three-dimensionally into the measurement chamber.

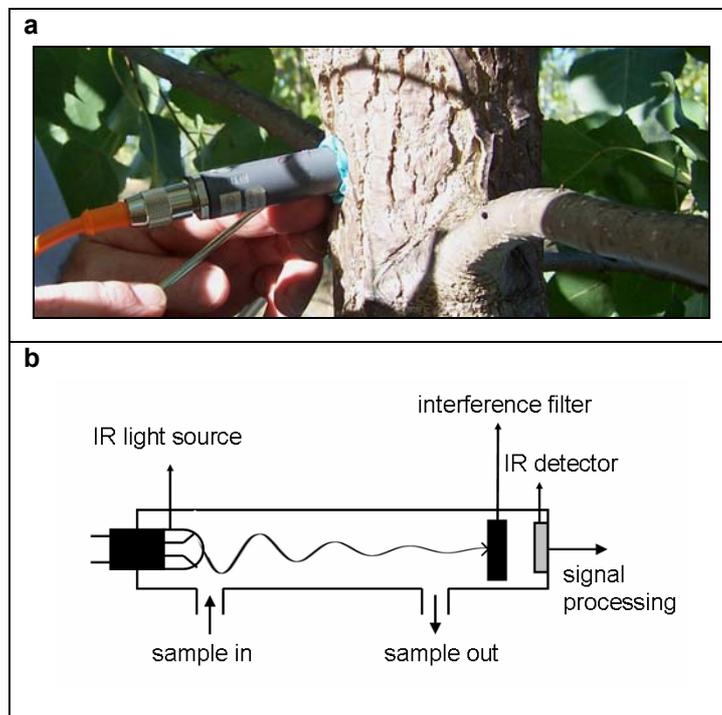


Figure 2.6: (a) Mounting of the non-dispersive infrared (NDIR) sensor in the stem of an eastern cottonwood. Flexible putty adhesive at the junction of the stem and sensor provides an airtight seal. (b) Schematic of the NDIR sensor.

The calibration procedure is similar as for the microelectrodes, except that it is performed at a constant temperature (25 °C). The response of the NDIR sensor to [CO₂] is linear. However, the sensor is affected by temperature and pressure due to the compressibility of gases (ideal gas law, Equation 2.3). When temperature and atmospheric pressure differ from 25 °C and 1013 hPa, a temperature and pressure correction provided by the Vaisala company (part of their patented technology, Patent No. US5646729) needs to be applied (Figure 2.7).

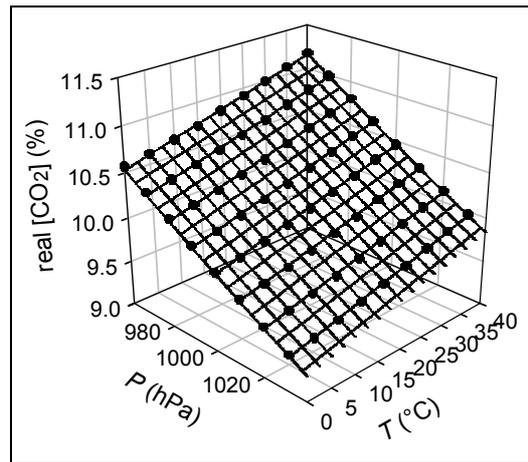


Figure 2.7: Real CO_2 concentration ($[\text{CO}_2]$) at different atmospheric pressures (P) and temperatures (T) when measured $[\text{CO}_2]$ by the non-dispersive infrared sensor is 10%. At 25 °C and 1013 hPa, real $[\text{CO}_2]$ is equal to measured $[\text{CO}_2]$.

2.3.2 Results and discussion of $[\text{CO}_2]$ measuring techniques

2.3.2.1 Gas extraction and gas chromatography

Figure 2.8 displays the seasonal course of the mean xylem $[\text{CO}_2]$ measured at four heights in a young beech and oak stem under controlled environmental conditions. Xylem $[\text{CO}_2]$ ranged between 1.2 and 7.2%, which is in the range of previously reported values (Table 1.1). A seasonal trend in $[\text{CO}_2]$ could not be observed in the beech, whereas the oak showed a peak in mid summer. However, standard errors were large and complicated interpretation of the measurements. Several difficulties were encountered using this technique. First, the tubes were sometimes (partially) filled with sap, which made gas extraction impossible. Second, the large difference between the samples of the same tree presumes that leaks were induced into the samples. Although the sample volume was only one fifth of the tube volume, a small leak may cause large errors due to the large difference between $[\text{CO}_2]$ in the stem and $[\text{CO}_2]$ of the surrounding air. To be able to exclude data from leaky samples, it is recommended that more than four samples are taken. However, this was not possible in this study due to the small size of the trees. Another disadvantage of the technique is that the calibration of the GC is a laborious task which needs to be redone every day.

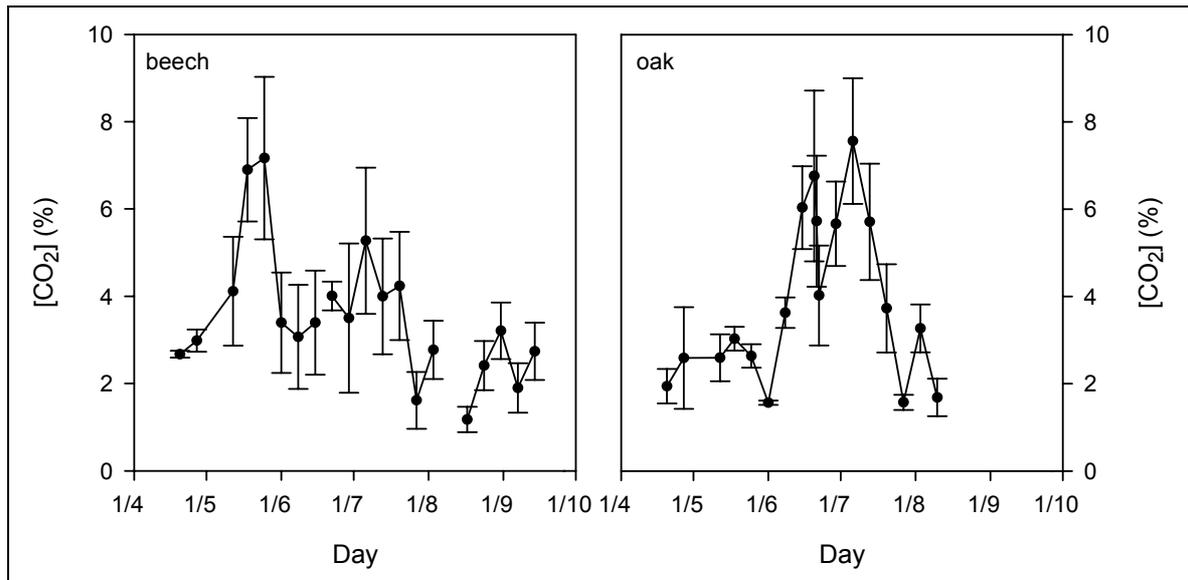


Figure 2.8: Mean CO_2 concentrations in the gaseous phase of the xylem ($[\text{CO}_2]$) of a young beech and oak measured by gas extraction and gas chromatography. The trees were grown in a growth chamber under constant air temperature and a 12/12-h photoperiod. Error bars represent \pm standard error, $n = 4$.

2.3.2.2 CO_2 microelectrode

Xylem $[\text{CO}_2]$ of the young trees as measured by the CO_2 microelectrodes in the growth chamber exhibited a diurnal pattern on most days. For example, in Figure 2.9a $[\text{CO}_2]$ is slightly higher during the light period than during the dark period, corresponding with a slightly higher air temperature during the light period. Another typical diurnal pattern is displayed in Figure 2.9b. During the light period, $[\text{CO}_2]$ showed a decrease during the light period and an increase in the dark period. The microelectrode response was sensitive and rapid enough to detect changes in $[\text{CO}_2]$ when measurements were recorded at 5-min intervals. However, several problems were encountered in the use of the microelectrodes. First, when the sensors were inserted in the trees after calibration, the response sometimes shifted. It was found that air bubbles in the $\text{NaHCO}_3/\text{NaCl}$ electrolyte solution were the cause of this shift. In that case, the sensors had to be refilled with the solution and re-calibrated. The calibration coefficients of each microelectrode could greatly differ between two successive calibrations. Another problem is drift in microelectrode response over time. This was observed as a steady increase or decrease in the potential difference over time. It is probably caused by water molecules diffusing into the electrolyte solution or contamination of the membrane (McGuire and Teskey 2002). This drift was sometimes difficult to distinguish from real changes in $[\text{CO}_2]$. We assumed that when the changes in $[\text{CO}_2]$ were observed in all microelectrodes, they were caused by actual changes in $[\text{CO}_2]$ and not by drift. The life span of the membrane and electrolyte solution was unpredictable. Sometimes the sensors exhibited drift after several days, sometimes after several weeks. It was impossible to monitor $[\text{CO}_2]$ during a whole growing season without replacing the housings and re-calibrating. In

conclusion, the microelectrodes are suitable for the assessment of diurnal dynamics in $[\text{CO}_2]$, but fail to monitor long-term changes in $[\text{CO}_2]$.

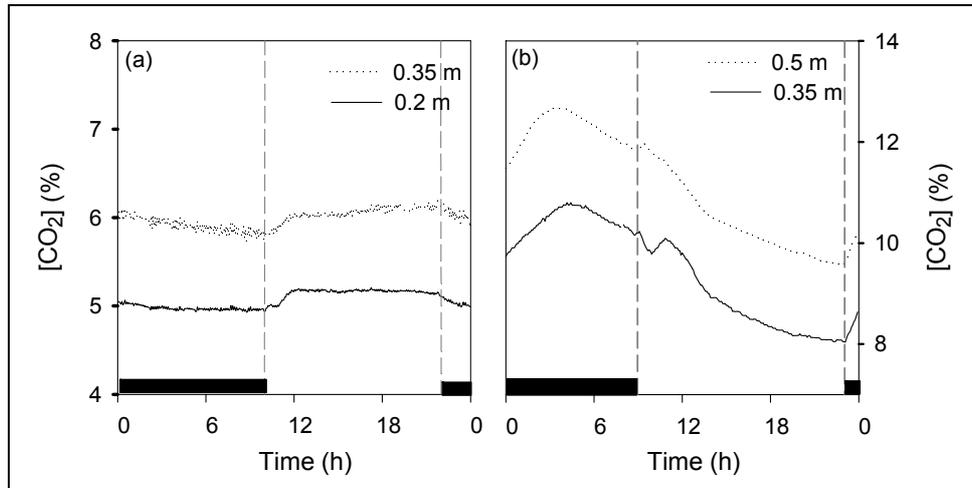


Figure 2.9: Typical diurnal patterns of the CO_2 concentration in the gas phase of the xylem ($[\text{CO}_2]$) as measured by CO_2 microelectrodes. Measurements were performed in growth chambers (a) on a young oak at two heights (0.2 and 0.35 m above the ground) and (b) on a young eastern cottonwood at two heights (0.35 and 0.5 m above the ground). Beginning and end of each dark period are indicated by black boxes and vertical dashed lines.

2.3.2.3 Solid state non-dispersive infrared sensor

Xylem $[\text{CO}_2]$ in the eastern cottonwood tree as measured by the NDIR sensor exhibited a clear diurnal pattern on most days. On sunny days, xylem $[\text{CO}_2]$ was highest during the night and decreased during the day (Figure 2.10a). However, depending on climatic variables such as temperature and rain, the diurnal pattern was sometimes less clear (Figure 2.10b). The NDIR sensors were stable and reliable and their response was sensitive and rapid enough to detect changes in $[\text{CO}_2]$ when measurements were recorded at 5-min intervals. Unlike the CO_2 microelectrodes, the sensors exhibited no drift and frequent re-calibration was not necessary. The calibration coefficients of two successive calibrations were very similar, which gave more confidence in interpreting the absolute $[\text{CO}_2]$ values. Hence, besides diurnal dynamics, also long-term changes in xylem $[\text{CO}_2]$ can be monitored with these sensors. One disadvantage of the NDIR sensors is that they are relatively large, making it necessary to drill large holes into the stem. Therefore, the technique can not be applied on small trees.

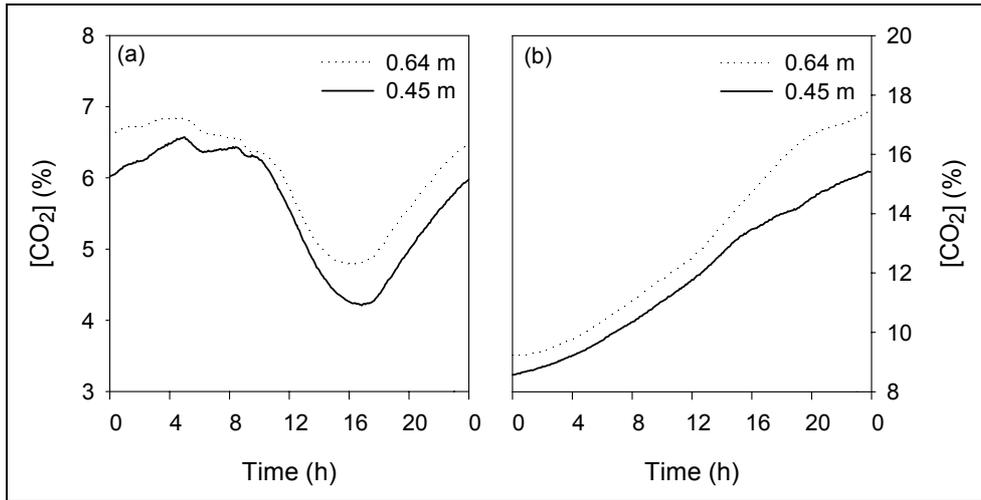
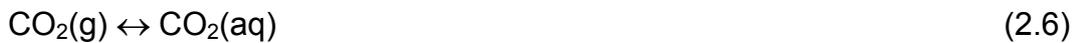


Figure 2.10: CO₂ concentrations in the gas phase of the xylem ([CO₂]) in eastern cottonwood stems at two heights (0.45 and 0.64 m above the ground) as measured by non-dispersive infrared sensors. Measurements were performed in a field near Athens, Georgia, USA (a) on a sunny day and (b) on a rainy day.

2.3.3 Conversion of [CO₂] to [CO₂*]

When water and air have a rather permanent boundary layer, an equilibrium is formed between the CO₂ concentration in the air and in the water. Gaseous CO₂ dissolves in water to form aqueous CO₂ (Stumm and Morgan 1996):



CO₂(aq) reacts with water:



Hence, the concentration of all products of CO₂ dissolved in water is:

$$[\text{CO}_2^*] = [\text{CO}_2](\text{aq}) + [\text{H}_2\text{CO}_3] + [\text{HCO}_3^-] + [\text{CO}_3^{2-}] \quad (2.10)$$

At normal ambient temperatures, [H₂CO₃] is negligible compared to [CO₂](aq) (Stumm and Morgan 1996). The equilibrium constants can be written as:

$$K_{\text{H}} = \frac{[\text{CO}_2](\text{aq})}{p\text{CO}_2} \quad (2.11)$$

$$K_1 = \frac{[\text{HCO}_3^-][\text{H}^+]}{[\text{CO}_2](\text{aq})} \quad (2.12)$$

$$K_2 = \frac{[\text{CO}_3^{2-}][\text{H}^+]}{[\text{HCO}_3^-]} \quad (2.13)$$

where K_H is Henry's law constant for CO_2 , K_1 and K_2 are acidity constants and $p\text{CO}_2$ is the CO_2 partial pressure above the water ($= [\text{CO}_2](g)$). From these equations, $[\text{CO}_2](aq)$, $[\text{HCO}_3^-]$ and $[\text{CO}_3^{2-}]$ can be calculated:

$$[\text{CO}_2](aq) = K_H \times p\text{CO}_2 \quad (2.14)$$

$$[\text{HCO}_3^-] = K_1 \times K_H \times p\text{CO}_2 / [\text{H}^+] \quad (2.15)$$

$$[\text{CO}_3^{2-}] = K_2 \times K_1 \times K_H \times p\text{CO}_2 / [\text{H}^+]^2 \quad (2.16)$$

For temperatures between 0 and 50 °C the constants K_H , K_1 and K_2 can be calculated with the following equations (Butler 1991):

$$K_H = 0.0114 + 0.0661 \times e^{-0.0433T} \quad (2.17)$$

$$K_1 = (2.5764 \times 10^{-7}) + (3.3742 \times 10^{-7}) \times (1 - e^{-0.0318T}) \quad (2.18)$$

$$K_2 = (2.3777 \times 10^{-11}) + (9.0041 \times 10^{-13}) \times T \quad (2.19)$$

where T is temperature (°C).

Assuming that the xylem sap can be approached as water, $[\text{CO}_2^*]$ of xylem sap can be calculated from $[\text{CO}_2]$ of the gas in contact with the sap. However, pH ($[\text{H}^+]$) and T affect the total amount of carbon dissolved in xylem sap (Equations 2.14 – 2.19, Figure 2.11).

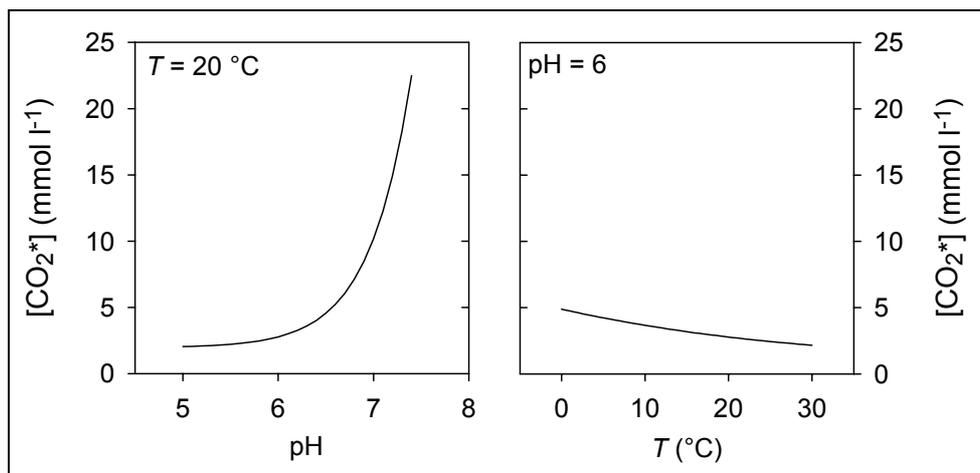


Figure 2.11: Effects of pH and temperature (T) on the concentration of all products of CO_2 dissolved in water ($[\text{CO}_2^*]$), when the CO_2 partial pressure above the water ($p\text{CO}_2$) is 5%.

Stem temperatures (T_{st}) were continuously monitored with copper-constantan thermocouples inserted in the stem near the holes. We are unaware of techniques for continuously monitoring of xylem sap pH. Problems in xylem sap sampling arise because the sap is under negative pressure when the tree is transpiring. Incision causes entry of air rather than leakage of xylem sap. The traditional method to obtain xylem sap consists of collecting detached pieces of a tree, putting them under pressure and collecting the sap that is squeezed out of the cut end (Schurr 1998). In this thesis, xylem sap was destructively sampled using two techniques. First, xylem sap was expressed from a twig with a pressure chamber (PMS Instruments, Corvallis, OR, USA) (Figure 2.12a). Second, sap was expressed with a vise from 5-mm diameter stem increment cores (Figure 2.12b). The pH of the expressed sap was measured with a solid state pH microsensor connected to a pH meter (Red-Line Standard sensor, Argus meter, Sentron, Roden, The Netherlands). Because sampling was destructive, pH measurements were performed after the experiments and it was assumed that the pH was constant during the experiments.

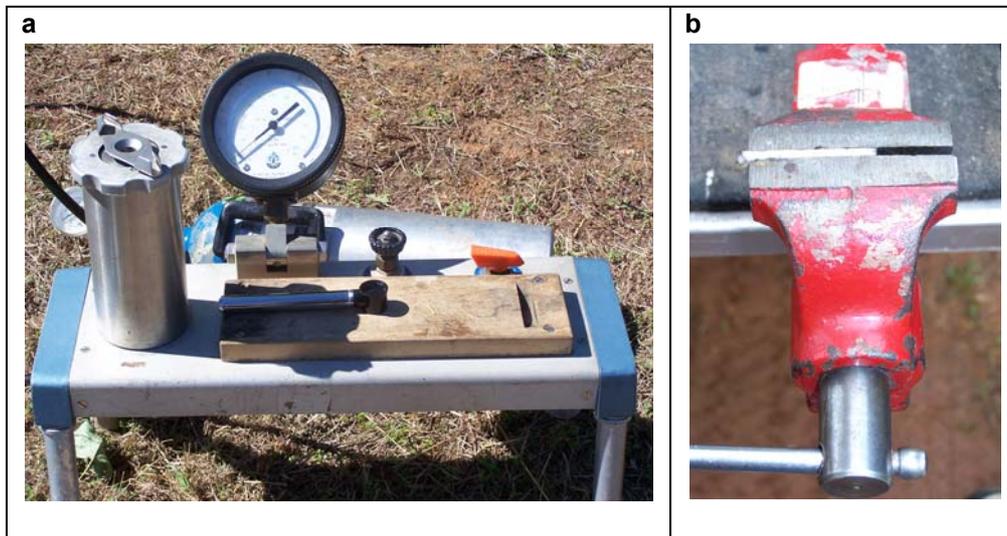


Figure 2.12: Instruments for expressing xylem sap: (a) a pressure bomb for expressing sap from excised twig; (b) a vise for expressing sap from increment cores.

The pH of the xylem sap measured in different species (*Fagus sylvatica*, *Quercus robur* and *Populus deltoides*) and under different growth conditions (growth chamber and field) ranged between 5.8 and 7. These values are in the same range as previously reported values for xylem sap pH (Table 2.1).

No significant difference between pH values obtained with the two methods was found. However, it has been reported that the xylem sap pH decreased with sampling height. Schill et al. (1996) found that over a distance of 5 m, xylem sap pH decreased by approximately one unit in two *Acer platanoides* L. trees. We only tested the two methods on small trees, not higher than 3 m. The distance between the stem cores and the sampled twigs was probably too small to detect significant differences in pH. In this thesis it was assumed that pH was constant during the measurement period.

However, xylem sap pH has been reported to vary seasonally. Glavac et al. (1990a,b) found that xylem sap pH in the stem of 35-year-old beech was highest during autumn and winter and lowest during summer. To our knowledge, it has never been investigated whether xylem sap pH in tree stems varies diurnally. If this would be the case, large errors in the calculation of xylem sap $[\text{CO}_2^*]$ would be introduced due to the high sensitivity of $[\text{CO}_2^*]$ to pH (Figure 2.11). Therefore, in this thesis, measured CO_2 concentrations in the gas phase of the xylem rather than calculated concentrations of all products of CO_2 dissolved in the xylem sap are given. Only in Chapter 7 the calculation of $[\text{CO}_2^*]$ was needed in order to estimate the total respiration rate (R_S) of a stem segment based on the mass balance approach (McGuire and Teskey 2004, paragraph 1.4).

Table 2.1: Reported values of xylem sap pH.

Reference	pH value	Species
Carter and Larsen 1965	5.6	<i>Pinus taeda</i> L.
Glavac et al. 1990a	4.5 – 6.8	<i>Fagus sylvatica</i> L.
Glavac et al. 1990b	4.8 – 6.5	<i>Fagus sylvatica</i> L.
Gollan et al. 1992	5.6 – 7.2	<i>Helianthus annuus</i> L.
Stringer and Kimmerer 1993	6.3	<i>Populus deltoides</i> Bartr. ex Marsh.
Schurr and Schulze 1995	5.4 – 6.2	<i>Ricinus communis</i> L.
Schill et al. 1996	6.1 – 7.4	<i>Acer platanoides</i> L.
Levy et al. 1999	6.4	<i>Betula pendula</i> Roth
Levy et al. 1999	6.8	<i>Musanga cecropioides</i> R. Br. ex Tedlie
Levy et al. 1999	5.2	<i>Distemonanthus benthamianus</i> Baill.
McGuire and Teskey 2002	5.4 – 6.8	<i>Liriodendron tulipifera</i> L. and <i>Quercus alba</i> L.
McGuire and Teskey 2004	6.5	<i>Fagus grandifolia</i> Ehrh.
McGuire and Teskey 2004	6.0	<i>Platanus occidentalis</i> L.
McGuire and Teskey 2004	5.6	<i>Liquidambar styraciflua</i> L.
Teskey and McGuire 2007	5.6 – 6.0	<i>Platanus occidentalis</i> L.

2.4 Sap flow measurements

Since the transpiration stream may transport dissolved CO_2 into and out of the xylem of a stem segment (paragraph 1.3.3), measurement of the sap flow rate (f_s) through the segment is indispensable to get a better understanding of the daily dynamics in E_{CO_2} and to quantify the CO_2 transport flux (F_T) for estimating total stem R_S (Equation 1.4). In this thesis, two types of sensors were employed, both based on heat transport: (1) Dynagage sap flow sensors (Model SGB16-WS, Dynamax Inc., Houston, TX, USA) based on the constant power heat balance principle and (2) thermal dissipation probes (Models TDP-30 and TDP-50, Dynamax Inc., Houston, TX, USA) (Granier 1985, 1987) (Figure 2.13).

The heat balance sensor consists of a flexible heater wrapped around a stem segment (Figure 2.13a). Thermocouple pairs connected in series are embedded in a cork band surrounding the heater to form a thermopile. One junction of each pair is positioned on the inner surface of the cork, the other junction at the outer surface, so

that the thermopile measures the radial temperature gradient away from the heater (Steinberg et al. 1989). Two other thermocouple pairs are positioned against the stem surface, one junction of each pair above the heater and one below (Steinberg et al. 1990), so that the thermocouple pairs measure the axial temperature gradient. A uniform amount of heat is applied to the entire stem circumference and the mass flow rate of sap (f_s ; g h^{-1}) is obtained from the balance of the axial and radial fluxes of heat into and out of the heated segment (Sakuratani 1981, Baker and van Bavel 1987).

The thermal dissipation probe consists of two needles, inserted radially into the stem, one needle placed 40 – 100 mm above the other (Figure 2.13b). The upper needle contains a heater and a thermocouple junction that is referenced to another junction in the lower needle (Granier 1985, 1987). Constant power is applied to the heater and the temperature difference between the needles is related to the sap flow velocity (v_s ; cm h^{-1}) around the needles. As sap flow increases, heat is dissipated more rapidly and the temperature difference decreases. Mass flow rate of sap is then calculated by multiplying with the cross-sectional area of the conducting sapwood.

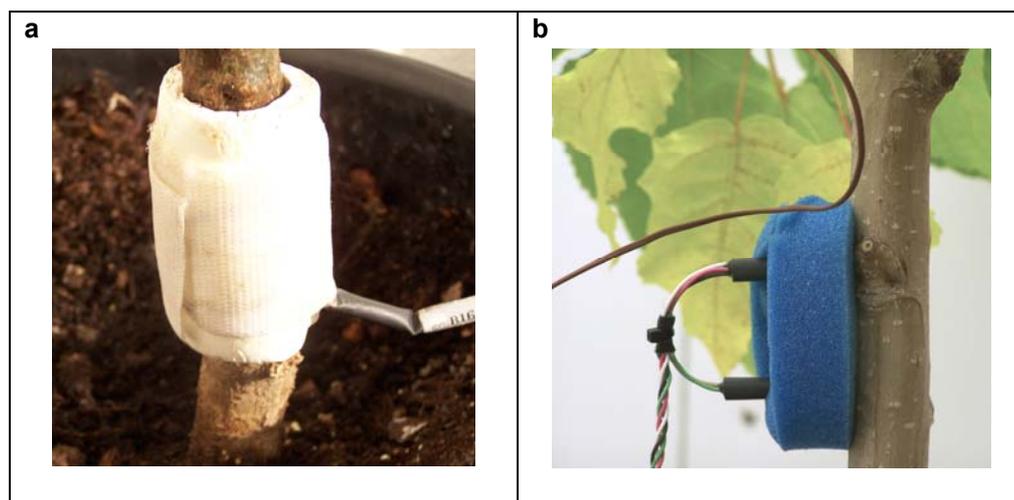


Figure 2.13: (a) Dynagage sap flow sensor mounted on an oak stem; (b) thermal dissipation probe mounted on an eastern cottonwood stem. After installation the sensors were covered with several layers of aluminium foil to ensure sufficient thermal insulation.

A major advantage of the heat balance sensor compared to the thermal dissipation probe is that it provides a direct measurement of the sap flow rate through the entire stem segment, whereas the thermal dissipation probe measures sap flow only at one point in the sapwood and it is assumed that sap flow is uniform across the sapwood. However, large radial and circumferential variability in v_s has been observed in several studies (Dye et al. 1991, Čermák et al. 1992, Becker 1996, Philips et al. 1996, Loustau et al. 1998, Oren et al. 1999), resulting in systematic errors in estimates of f_s (Nadezhdina et al. 2002, Ford et al. 2004). In the field study, v_s was measured at two different depths (1.5 and 2.5 cm) with two thermal dissipation probes (Models TDP-30 and TDP-50) in cottonwood stems ranging in diameter between 7.7 and 8.9 cm. It was found that v_s was significantly lower at 2.5 cm than at

1.5 cm depth (Figure 2.14). Mean v_s of the two probes was used to calculate f_s , which served as an approximation of the real f_s .

A major disadvantage of the heat balance sensor compared to the thermal dissipation probe is that it is only suitable for small stems (Smith and Allen 1996) precluding application in the larger cottonwood trees.

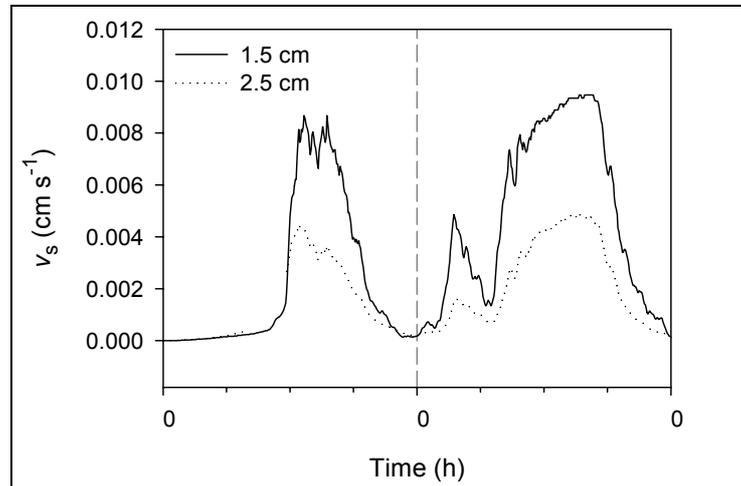


Figure 2.14: Two-day course of sap flow velocity (v_s) at two depths (1.5 and 2.5 cm) in an eastern cottonwood stem as measured by thermal dissipation probes. Measurements were performed on sunny days in a field near Athens, Georgia, USA.

2.5 Stem diameter measurements

Stem diameter variations reflect the irreversible tissue growth and the reversible changes in hydration of the stem tissue (Kozlowski 1972). Typical changes in stem hydration are the depletion of stem water reserves during the day and the replenishment in the evening, as a consequence of sap flow. Hence, diameter variations comprise information on both the growth rate and the water status of the stem. Both variables are of interest in this thesis, since they affect the respiration rate of the living stem tissues (paragraph 1.2.3.2). Stem diameter variations were measured with linear variable displacement transducers (LVDTs) (Model LBB, 375-PA-100 and transducer bridge 8C-35, Schaevitz, Hampton, VA, USA). The LVDTs were fixed on the stem with custom-made stainless steel holders (Figure 2.15).

Figure 2.16 shows a typical course of stem diameter change. The variation in stem diameter reflects both the radial growth of the stem and changes in stem tissue water content. A parameter characterizing tissue growth is the daily growth (DG), corresponding to the difference between two successive daily maximum values of D (just before onset of shrinkage). A parameter characterizing the stem water status is the maximum daily shrinkage (MDS), corresponding to the difference between the maximum and minimum values of D during a day (Figure 2.16).



Figure 2.15: A linear variable displacement transducer (LVDT) installed on a young beech stem and supported by a custom-made stainless steel holder.

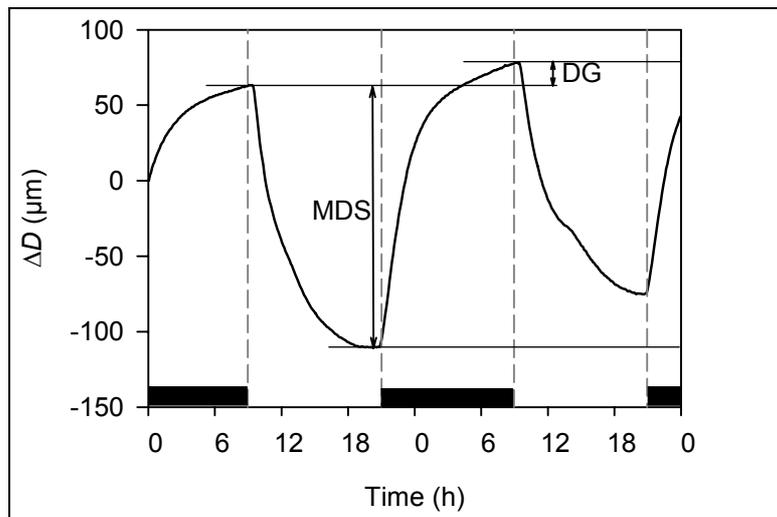


Figure 2.16: Two-day course of stem diameter change (ΔD) in a young oak tree as measured by a linear variable displacement transducer. The stem diameter at the start of measurement was 18.9 mm. Measurements were performed in a growth chamber with a 12/12 h photoperiod and constant air temperature. Beginning and end of each dark period are indicated by black boxes and vertical dashed lines. DG = daily growth; MDS = maximum daily shrinkage.

2.6 Stem water potential measurements

Another indicator of the water status of the stem is the stem water potential (Ψ_{st}). Two instruments were applied in this thesis to determine Ψ_{st} : (1) a pressure bomb (PMS Instruments, Corvallis, OR, USA) and (2) a thermocouple psychrometer (Model PST-55, Wescor, Logan, UT, USA).

The pressure bomb (Figure 2.12a) is the traditional instrument used for measuring water potentials of excised leaves or twigs. When the plant material is cut, the xylem sap is withdrawn from the cut surface due to the tension in the plant material. The leaf or twig is placed in the chamber with the cut surface protruding through a rubber seal. The pressure in the chamber is then raised until sap appears at the cut surface. The positive pressure needed to express the sap is equal to the tension in the plant material before excision. If the osmotic potential of the xylem sap is negligible, the

tension is approximately equal to the water potential (Ψ). When f_s is zero (during the dark), Ψ of an excised leaf is assumed to correspond with Ψ of the stem because there are no transpiring leaves creating a tension gradient along the tree. However, determining Ψ_{st} during the light is more complicated. Under transpiration, a tension gradient develops, so that Ψ of the leaves is more negative than Ψ_{st} . To eliminate this tension gradient, transpiration of a leaf was artificially prevented by enclosing it in an aluminium envelope 2 h before measurement. Assuming zero f_s in the petiole of the leaf, Ψ of the excised leaf corresponds with Ψ_{st} (McCutchan and Shackel 1992).

The thermocouple psychrometer was originally developed for measuring soil water potential. The sensor measures the relative humidity (RH) of a small sample of air that has been allowed to equilibrate with the soil (or the plant material in our case). RH is related to Ψ (Pa) (Spanner 1951):

$$\Psi = \frac{R_u \times T}{V_w} \times \ln \frac{e}{e^0} \quad (2.20)$$

where R_u is the universal gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$), T the absolute temperature (K), V_w the molar volume of water ($1.8 \times 10^{-5} \text{ m}^3 \text{ mol}^{-1}$) and e / e^0 the relative humidity expressed as a fraction. To measure Ψ_{st} , the thermocouple psychrometer sensor was mounted in a hole in the stem (4 mm diameter and 1 cm length) and sealed to the stem surface with flexible putty adhesive (Figure 2.17)



Figure 2.17: A thermocouple psychrometer sensor inserted in an eastern cottonwood stem. The sensor is sealed to the stem surface with flexible putty adhesive.

The thermocouple psychrometer has several advantages compared to the pressure bomb: (1) measurements are non-destructive, whereas the pressure bomb requires excision of leaves, (2) Ψ of the stem is measured *in situ*, whereas the pressure bomb measures Ψ of excised leaf petioles and (3) measurements are continuous. However, several problems were encountered when using this sensor. We found that the response to changes in Ψ_{st} was sometimes very slow, and that at high Ψ_{st} , the sensor was unable to detect changes in Ψ_{st} . We compared thermocouple psychrometer measurements of Ψ_{st} with LVDT measurements of stem diameter and found that at the onset of the light period the decrease in Ψ_{st} was much later than the

decrease in stem diameter. The difference in response time was most pronounced when Ψ_{st} was high (Figure 2.18). When the lights were switched off, the response time of the thermocouple psychrometer was similar as that of the LVDT. Another major disadvantage of the thermocouple psychrometer is that the sensor exhibited a complete failure under non-stable temperature conditions, like in the field.

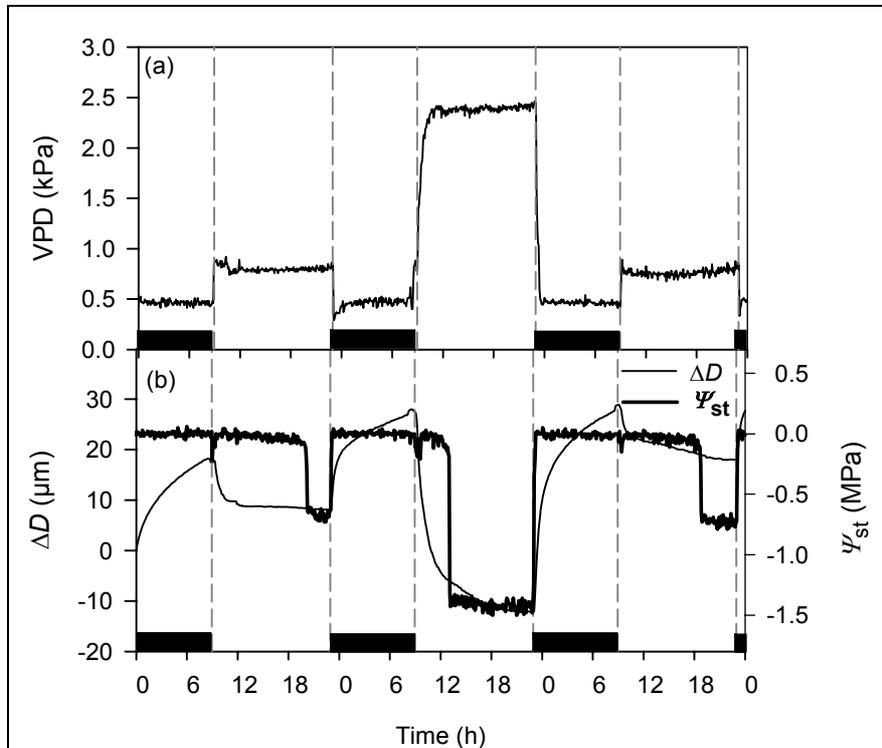


Figure 2.18: (a) Three-day pattern of vapour pressure deficit (VPD) in a growth chamber with a constant dark/light regime and different regimes of relative humidity (85% during dark, 75 % during light on days 1 and 3, 35% during light on day 2); (b) Stem diameter changes (ΔD) and stem water potential (Ψ_{st}) in an eastern cottonwood tree grown in the chamber, measured with a linear variable displacement transducer and a thermocouple psychrometer. Beginning and end of each dark period are indicated by black boxes and vertical dashed lines.

2.7 Conclusions

In this chapter we attempted to merge useful information on measuring techniques for different variables of interest when studying stem respiration: stem CO_2 efflux rate, xylem CO_2 concentration, pH, sap flow rate, stem diameter changes and stem water potential. For measuring xylem CO_2 concentration, sap flow rate and water potential, alternative techniques were applied throughout the thesis. This chapter gave an overview of these techniques, together with their major virtues and shortcomings.

Particular attention was paid to measuring techniques for xylem CO_2 concentrations, since some of the applied techniques have only recently been introduced in tree physiological studies. We found that the most reliable measurements were obtained

with the non-dispersive infrared sensors. Unlike the CO₂ microelectrodes, these sensors were able to function for long periods of time without exhibiting drift problems. However, a disadvantage is that the dimensions of the non-dispersive infrared sensor are relatively large, so that they can not be used in small tree stems. To be able to calculate how much of the xylem CO₂ can be transported with the sap, it has to be identified how much CO₂ is dissolved in the xylem sap. Therefore, temperature and pH of the xylem sap need to be known. We tested two destructive sampling techniques for collecting xylem sap: excision of twigs in combination with collection of sap with the pressure bomb and taking stem cores in combination with collection of sap with a vise. Similar results were obtained.

To quantify the CO₂ transport flux in the xylem, also xylem sap flow rate needs to be measured. The most appropriate technique is the heat balance method, because it gives a direct measure of the sap flow rate through the entire stem segment. Thermal dissipation probes measure sap flow velocity at only one point in the sapwood, thereby assuming that sap flow velocity is uniform across the sapwood. However, measuring sap flow velocity at two depths revealed that this assumption is wrong and, hence, that applying this technique may introduce errors in estimating the sap flow rate.

A variable that may affect stem respiration is the stem water status. To achieve information on this variable, stem water potential was measured with the pressure bomb and with the thermocouple psychrometer. Although the thermocouple psychrometer had several advantages over the pressure bomb (non-destructive and continuous measurements and direct measurement of the water potential of the stem instead of the leaf petiole), the sensor showed sometimes a very slow response and exhibited complete failure in environments with fluctuating temperatures.

Changes in stem water status can also be monitored with linear variable displacement transducers. They measure stem diameter measurements, which reflect both changes in stem hydration and radial stem growth. Since stem respiration rates are affected by both the growth rate and the water status of the stem, stem diameter measurements present a useful tool in stem respiration studies.

Chapter 3

Report on non-temperature related variations in CO₂ efflux rates from young tree stems in the dormant season

Adapted from : Saveyn A, Steppe K and Lemeur R (2006) Report on non-temperature related variations in CO₂ efflux rates from young tree stems in the dormant season. *Trees – Structure and Function*, published online (DOI 10.2007/s00468-0060099)

Abstract

Respiration rates are reported to increase exponentially with temperature. Respiration rates of woody tissues are commonly measured as CO₂ efflux rates (E_{CO_2}) from that tissue. However, this study describes clear variations in stem E_{CO_2} that were not related to temperature for the case of a young beech (*Fagus sylvatica* L.) and oak (*Quercus robur* L.) tree during the dormant season. The CO₂ concentration ([CO₂]) in the xylem of the beech tree showed similar temperature-independent variations. The trees were grown in a growth chamber in which radiation patterns and temperature were kept constant. E_{CO_2} was measured with an IRGA connected to cuvettes surrounding a stem segment. Xylem [CO₂] was measured *in situ* using a CO₂ microelectrode. Depressions in E_{CO_2} and [CO₂] occurred during the light period, despite equal stem temperatures in the light and dark period. Explanations found in literature for discrepancies in the exponential relationship between temperature and E_{CO_2} are the influence of (1) sap flow or (2) decreased cell water content. However, (1) the variations were observed in the dormant season, when no sap flow was observed yet, and (2) reduced cell water content was not likely to be apparent as differences in stem transpiration rates between the dark and light period were not significant. Hence, previously formulated theories failed to explain our results. This work therefore provides a new ground for discussion on other possible causes of daytime depressions in E_{CO_2} . One might be the re-fixation of respired CO₂ by cortical photosynthesis in the stem parts adjacent to the stem segment enclosed by the cuvette.

3.1 Introduction

The strong influence of temperature (T) on respiration rates of plant tissues has been well documented. Respiration rate at a given temperature T is frequently calculated by means of an exponential equation in terms of Q_{10} (the relative increase in respiration rate with a 10 °C increase in air or tissue T) and the respiration rate at a reference temperature T_r (e.g., Lavigne and Ryan 1997, Damesin 2003) (Equation 1.2). For a wide variety of plant organs Q_{10} ranges from 1.6 to 3, but centres about 2 in the physiologically relevant T range (Amthor 1989). Q_{10} reported for woody plants appears to vary less, but might be slightly higher (Ryan 1991b). A Q_{10} of 2 is often used in the development of natural ecosystem models involving carbon or energy exchange. This assumption has been generally accepted because of the convenience that it offers and the scarcity of diurnally and seasonally collected ecosystem respiration data (Edwards and McLaughlin 1978).

In some cases, good correspondence has been found between measured CO_2 efflux rates (E_{CO_2}) and T (e.g., Levy and Jarvis 1998, Maier et al. 1998). However, in other cases a relationship between measured E_{CO_2} and T was less clear or could not be even found. Kaipiainen et al. (1998) failed to find in summer any correlation between measured CO_2 efflux rates in trunks of *Pinus sylvestris* L. and the pattern of daily T . Gansert (2004) observed daytime reductions in E_{CO_2} in branches of *Betula ermanii* Cham. and *B. pendula* Roth compared to nighttime E_{CO_2} at a given T . A possible explanation for the observed depressions in E_{CO_2} is the influence of sap flow. Given the high solubility of CO_2 in water, it seems very probable that a part of the CO_2 evolved by the respiring cells of woody tissues is dissolved in the sap and is transported with the transpiration stream instead of escaping through the bark (Negisi 1972, Ryan 1990, Sprugel 1990, Teskey and McGuire 2002). In that case, measurement of E_{CO_2} is not an ideal tool for quantifying actual respiration rates because it substantially underestimates the actual respiration.

However, Edwards and Wullschleger (2000) found that stem E_{CO_2} of *Quercus prinus* L. and *Acer rubrum* L. trees was not altered by diurnal patterns of sap flow and they suggested that depressions in E_{CO_2} might be an indication of actual depressions in respiration rates. Edwards and McLaughlin (1978) observed daily cycles in stem E_{CO_2} of *Liriodendron tulipifera* L. and *Quercus alba* L. that were completely in opposite phase with temperature-based model predictions. Concentrations of reducing sugars in the stem followed a similar diurnal pattern as E_{CO_2} . It was suggested that by regulating the supply of reducing sugars available for synthesis, trees could shift some of their metabolic activities to those parts of the day when moisture availability is higher, i.e., at night. Little (1975) has shown that drought stress directly reduces rates of synthesis in *Abies balsamea*

(L.) Mill. trees. Moreover, according to Lockhart's model (1965), cell elongation does mainly occur at night, in relation to an improved water status and consequently a higher turgor pressure. Hence, reduced respiration because of low substrate availability and low turgor pressure may at least partly explain midday depressions in stem E_{CO_2} .

In this study, we report for the first time temperature-independent variations in stem E_{CO_2} for a young *Fagus sylvatica* L. and *Quercus robur* L. tree during the dormant season. Compared to the dark period, significant reductions in E_{CO_2} were observed during the light period. The CO₂ concentration ([CO₂]) in the xylem of the beech tree showed similar temperature-independent variations. The objective of this study was to demonstrate that previously formulated theories in literature for daytime depressions in E_{CO_2} failed to explain our results as neither sap flow, nor water deficit were observed in the tree stems. This study therefore aims at opening a new ground for discussion on other possible causes of temperature-independent variations in stem E_{CO_2} .

3.2 Materials and methods

3.2.1 Plant material and growth chamber

A 3-year-old beech tree (*Fagus sylvatica* L.) and oak tree (*Quercus robur* L.), previously grown outdoors were planted at the end of February 2005 in 50 l containers, filled with a mix of sandy loam soil and potting mixture. The trees were 1.6 m high and the stem diameters at the soil surface were 17.1 and 19 mm, for beech and oak respectively. The trees were placed in a growth chamber with dimensions 2 x 1.5 x 2 m (height x width x length). The growth chamber allowed the control of radiation levels and air temperature. Light was supplied by densely packed fluorescent lamps ('TL'D 80, Philips Lighting NV, Eindhoven, Netherlands), producing photosynthetic active radiation (PAR) during the light period (from 0900 h until 2100 h) of approximately 470 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the top of the tree (measured with a quantum sensor, Model Li-190S, Li-COR, Lincoln, TE, USA) (Figure 3.1). Air temperature (T_a) was measured with a copper-constantan thermocouple (Omega, Amstelveen, Netherlands), installed at a height of 1.1 m between both trees. Relative humidity (RH) was measured with a capacitive RH sensor (Model HIH-3605-A, Honeywell, Morristown, NJ, USA) at the same height of the thermocouple. Water potential of the soil (Ψ_{so}) was measured with a tensiometer (Model SWT6, Delta-T, Cambridge, UK) at a depth of 20 cm. The trees were watered weekly and fertilized monthly with a NPK plus micronutrient mix (Substral, Sint-Niklaas, Belgium). The measurement period started on 29 March 2005 (day 88) and ended on 10 April 2005 (day 100). In that period the trees were leafless (dormant season). The experimental set-up used in this study focused on the simultaneous measurement of several ecophysiological variables

of relevance for the interpretation of the CO₂ efflux process. One tree of each species was used for this purpose. More trees could not be measured at the same time due to the extensive equipment needed to conduct this study.

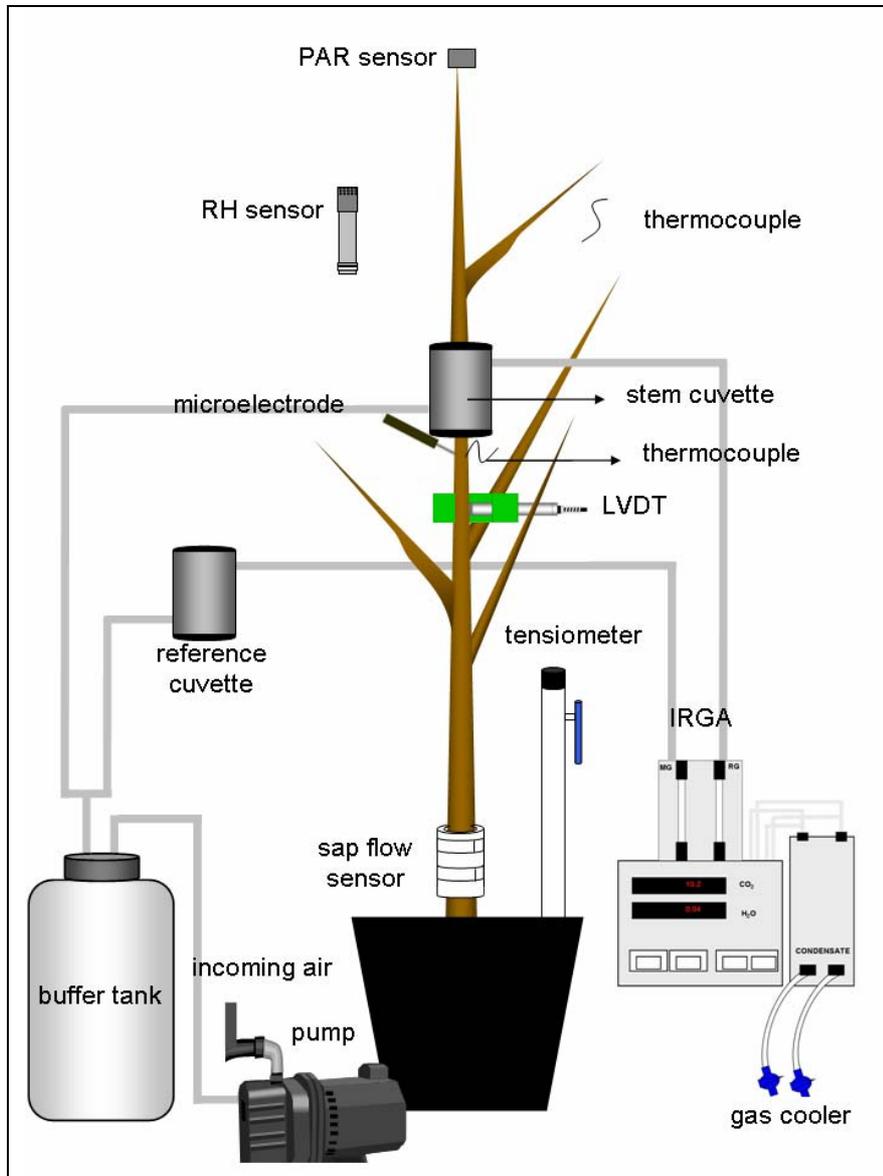


Figure 3.1: Schematic overview of the experimental setup in the growth chamber. Specifications about the sensors and devices are given in the text.

3.2.2 CO₂ and H₂O efflux measurements

CO₂ and H₂O exchange measurements were performed on a 13-cm long segment of each stem, approximately 0.7 m above soil level. The lower and upper diameter of the stem segment were 13.9 and 12.2 mm for beech and 11.0 and 10.2 mm for oak, respectively. The segments were enclosed in air-tight cylindrical acrylic cuvettes with a diameter of 6 cm. The cuvettes were covered with several layers of aluminium foil and black tape to exclude all light from reaching the stem tissue. In that way, photosynthesis by the stem tissue enclosed

in the cuvettes was prevented. Air from the growth chamber was mixed in a 50 l buffer tank to minimize short-term fluctuations in CO₂ and H₂O concentration before being pumped into the cuvettes by a membrane pump (Model N 86.KN 18, KNF Verder, Aartselaar, Belgium) at a flow rate of 1 l min⁻¹. The air leaving the cuvettes was first analyzed for H₂O concentration, and was then dried with a gas cooler (Model CG/G 73-4, Hartmann and Braun, Frankfurt, Germany) operating at 4 °C to remove most water vapour molecules, before being analyzed for CO₂ concentration. A Binos 100-4P IRGA (Fisher-Rosemount, Hanau, Germany), set up in differential configuration, measured the differences in CO₂ and H₂O concentration of the air of the stem cuvettes and a reference cuvette. The reference cuvette surrounded a plastic tube with a diameter of 12 mm, which is similar to the diameters of the stem segments, so that equal volumes and thus equal residence times were obtained. Signals from the IRGA were logged every 10 s during 12 min for each cuvette, and averaged values of the last 6 min were recorded by the data logger (HP 34970A, Hewlett Packard, Palo Alto, CA, USA). After each measurement a zero measurement of 6 min followed, in which air from the reference cuvette was led to the reference and measuring cell of the IRGA. Hence, two values per h for the CO₂ and H₂O efflux rates of each stem segment were obtained. Since only living cells are producing CO₂ and because in small trees the largest part of living cells is located in the external stem tissues (phloem and cambium) (Stockfors and Linder 1998), CO₂ efflux rates (E_{CO_2}) were expressed per unit of surface area. H₂O efflux rates (E_{H_2O}) were also expressed per unit of stem surface area.

3.2.3 Growth and sap flow measurements

Variations in stem diameter were measured using linear variable displacement transducers (LVDTs) (Model LBB, 375-PA-100 and transducer bridge 8C-35, Schaevitz, Hampton, VA, USA), placed 2 cm below the cuvettes. The LVDTs were attached by a custom-made stainless steel holder.

Sap flow rates were measured at the base of the stem with sap flow sensors based on the heat balance principle (Model SGB16-WS, Dynamax Inc., Houston, TX, USA). Sensor installation and calculation of sap flow rate (g h⁻¹) were performed according to the guidelines in the operation manual (van Bavel and van Bavel 1990). The gauges were wrapped in several layers of aluminium foil to ensure sufficient thermal insulation. Sheath conductance of the gauge was daily recalculated using minimum predawn values between 0400 h and 0700 h. The value for stem thermal conductance was taken from literature: for woody stems a value of 0.42 W m⁻¹ °C⁻¹ is considered as appropriate according to Steinberg et al. (1989).

3.2.4 Measurement of xylem CO₂ concentration

Xylem CO₂ concentration ([CO₂]) was measured *in situ* in the beech tree using the microelectrode technique described by McGuire and Teskey (2002). A CO₂ microelectrode (Model MI-720, Microelectrodes Inc., Bedford, NH, USA) was used for this purpose. To measure xylem [CO₂] in the beech tree, a 4-mm diameter hole was drilled 7 mm deep into the xylem at approximately 1 cm below the cuvette of the beech tree. A Teflon tube (3.5 mm inner diameter and 30 mm length) was tightly fitted into the hole and the tube was sealed to the tree using flexible putty adhesive (Terostat IX, Henkel, Heidelberg, Germany). The microelectrode was inserted in the tube and putty adhesive was used to provide a gas-tight seal. .

3.2.5 Temperature response of CO₂ efflux rate and xylem CO₂ concentration

For the measurement of stem temperature (T_{st}), copper-constantan thermocouples were inserted in the stem in 1-mm diameter holes with a depth of 7 mm, just below the stem cuvettes.

Because respiration is known to be strongly temperature dependent (see introduction) E_{CO_2} (expressed in $\mu\text{mol m}^{-2} \text{s}^{-1}$) was modelled by the following function:

$$E_{CO_2} = E_{CO_2}(20) \times Q_{10}^{\frac{T_{st}-20}{10}} \quad (3.1)$$

where $E_{CO_2}(20)$ is the CO₂ efflux rate at 20 °C, T_{st} the stem temperature (°C) and Q_{10} is the relative increase in respiration rate with a 10 °C increase in T (Damesin 2003). Parameters $E_{CO_2}(20)$ and Q_{10} were estimated by ordinary least squares in Matlab 6.5 (The Mathworks Inc., Natick, MA, USA). Each parameter was estimated separately for the dark period and the light period. Because xylem [CO₂] is likely to be related to the respiration rate, a similar equation was used to model the T response of [CO₂] (expressed in volume%):

$$[CO_2] = [CO_2](20) \times Q_{10}^{\frac{T_{st}-20}{10}} \quad (3.2)$$

where [CO₂](20) is the xylem CO₂ concentration at 20 °C and Q_{10} is the relative increase in [CO₂] with a 10 °C in T . The Q_{10} parameter is normally used to express an increase in a reaction rate. However, since [CO₂] and respiration rate are likely correlated, this parameter was also used in modelling [CO₂], in order to allow a comparison with the model of E_{CO_2} . Parameters were estimated separately for the dark and light period.

3.2.6 Data analysis

All signals from sensors and devices were logged at 10-s intervals using a data acquisition system (HP 34970A, Hewlett Packard, Palo Alto, CA, USA). A program was written (Hewlett-Packard VEE) to enable sensor measurements to be monitored continuously. All sensor signals were averaged over 360-s periods (except for CO₂ efflux measurements, see above) and recorded by a PC. Data were analysed using SigmaPlot 2001 (SPSS Inc., Chicago, IL, USA), SPSS 11.5 (SPSS Inc., Chicago, IL, USA) and Microsoft Excel XP (Microsoft Inc., Redmond, WA, USA).

3.3 Results

3.3.1 Microclimatological and physiological variables

Figure 3.2a shows a 4-day (day 94 – 98) example of the PAR at the top of the trees and T_{st} of the beech and oak tree. Mean PAR during the light period was $470 \mu\text{mol m}^{-2} \text{s}^{-1}$. T_{st} was nearly constant; $20.8 \pm 0.1 \text{ }^\circ\text{C}$ and $19.8 \pm 0.1 \text{ }^\circ\text{C}$ for beech and oak, respectively. The lower T_{st} of oak compared to beech might be due to higher latent heat losses (see below). Figure 3.2b displays RH in the growth chamber and Ψ_{so} . Mean RH for the period day 94 – 98 was $39 \pm 1.9\%$. Ψ_{so} remained nearly constant (except when the tree was watered on day 98) at a high level ($-12.3 \pm 0.8 \text{ hPa}$).

Different physiological variables of the trees were measured. During the measurement period no sap flow or diameter fluctuations occurred (data not shown). In Figure 3.2c E_{CO_2} of the beech and oak stem are displayed. E_{CO_2} is rather low because, in this period, the trees were still leafless and radial stem growth did not yet occur. Hence, respiration was only due to maintenance processes. E_{CO_2} shows a diurnal pattern, with the highest rates occurring in the dark period. Two-sample *t*-tests were conducted to estimate the statistical significance of differences in E_{CO_2} between the light and dark period. For both trees, differences are significant ($p < 0.01$). Values of E_{CO_2} of the oak tree were significant lower than those of the beech tree, but the reason for this remains unclear.

In Figure 3.2d, the water losses of the stem segments (E_{H_2O}) are displayed. Mean values were $0.26 \pm 0.02 \text{ mg m}^{-2} \text{ s}^{-1}$ and $1.04 \pm 0.05 \text{ mg m}^{-2} \text{ s}^{-1}$ for the beech and oak tree, respectively. Differences in E_{H_2O} between the dark period and the light period are not significant ($p > 0.01$). It is however, nor clear, why E_{H_2O} of the oak tree was significant higher than that of the beech tree.

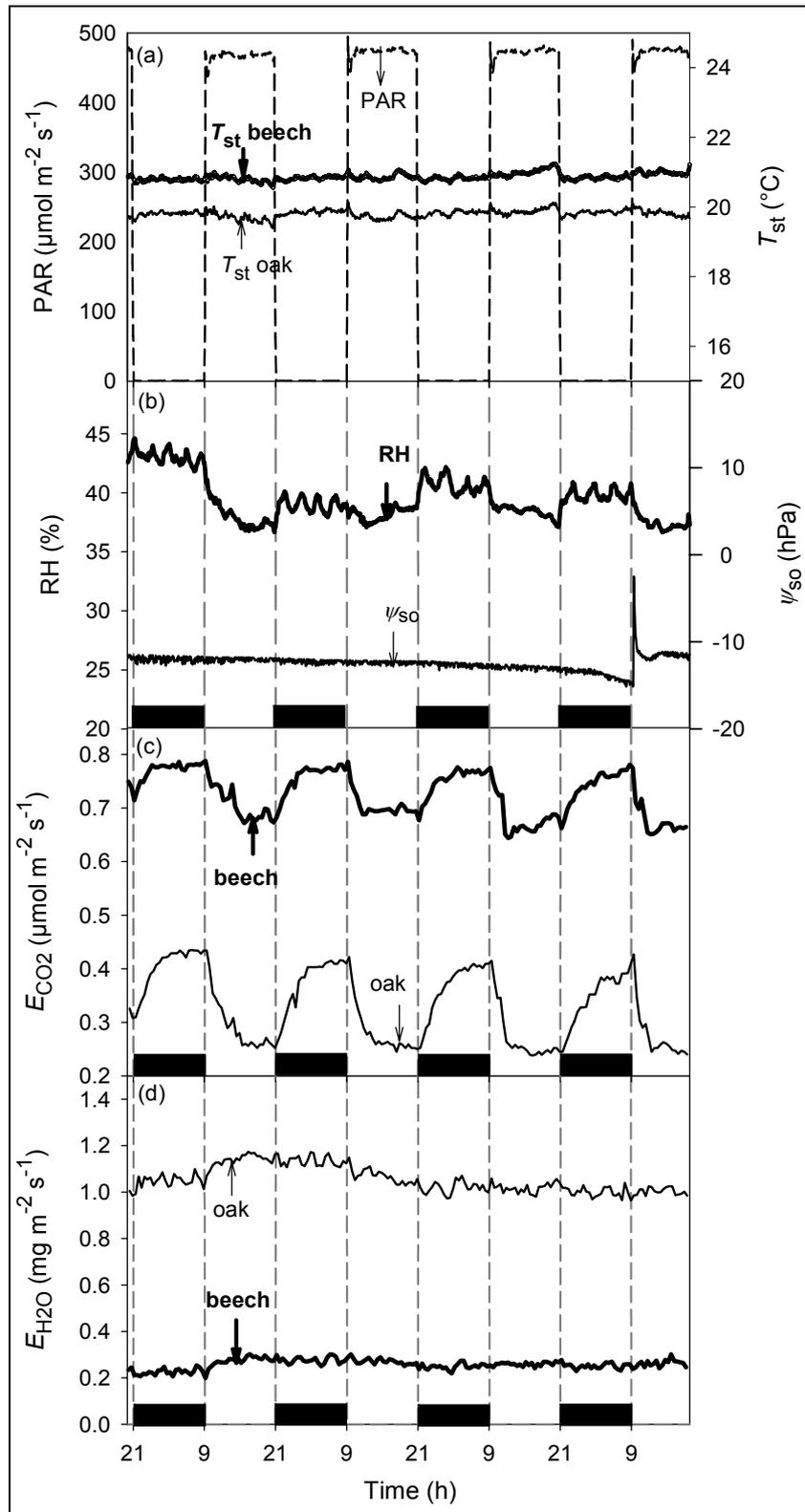


Figure 3.2: Patterns of the microclimatological variables in the growth chamber and the physiological responses of the young beech and oak tree in the period day 94 – 98 (dormant season): (a) photosynthetic active radiation (PAR) at the tree top level and stem temperature (T_{st}) of the beech and oak tree; (b) relative humidity of the air (RH) and water potential of the soil (Ψ_{so}); (c) CO_2 efflux rate (E_{CO_2}) of a stem segment of the beech and oak; (d) transpiration rate ($E_{\text{H}_2\text{O}}$) of the stem segment of the beech and oak tree. Beginning and end of each dark period are indicated by black boxes and vertical dashed lines.

Figure 3.3 shows the diurnal course of xylem [CO₂] in the beech tree for the period day 94 - 98. This variable ranges between 2.45% and 2.53% and shows a diurnal pattern with the highest values occurring in the dark period. Differences in xylem [CO₂] between the dark and light period are significant ($p < 0.01$). Diurnal fluctuations in [CO₂] correspond well with fluctuations in E_{CO_2} . The two variables are linearly correlated. Hence, it is demonstrated that a lower E_{CO_2} during the light period actually corresponded with a lower internal [CO₂].

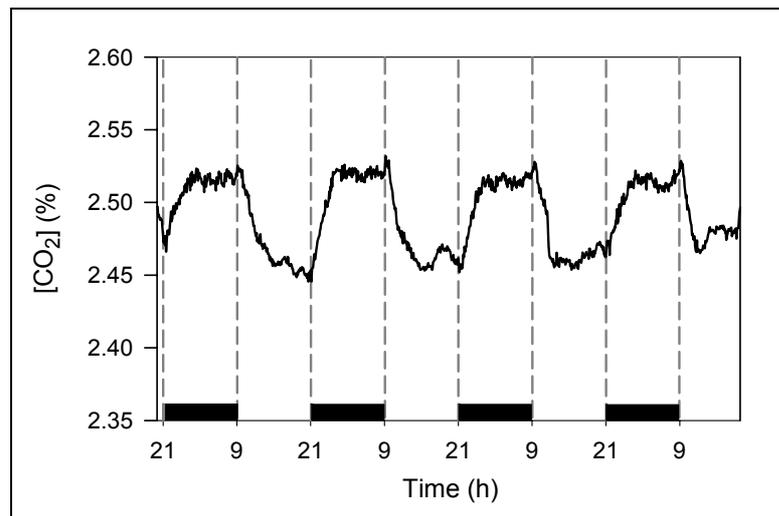


Figure 3.3: Time course of the xylem CO₂ concentration ([CO₂]) in the young beech tree for the period day 94 – 98. Beginning and end of each dark period are indicated by black boxes and vertical dashed lines.

3.3.2 Temperature response of CO₂ efflux rate and xylem CO₂ concentration

Fitting Equation 3.1 to measured E_{CO_2} for the period day 94 – 98 yielded very low coefficients of determination ($R^2 = 0.12$ and 0.14 for beech and oak, respectively). Moreover, estimated Q_{10} for the beech tree was 0.14 , which implicates that E_{CO_2} decreased with increasing T_{st} . However, since standard errors of estimated parameters were higher than parameter values, no conclusions about Q_{10} could be drawn. On day 99, T_a was altered stepwise during the light period and the same was performed in the dark period. Figure 3.4 displays the time course of T_a , T_{st} and E_{CO_2} on day 99. T responses of E_{CO_2} were considered separately for the dark and the light period.

A clear relationship could be found between T_{st} and E_{CO_2} for both the dark and the light period. In Figure 3.5a E_{CO_2} versus T_{st} is shown separately for the light and the dark period. E_{CO_2} exhibited a hysteresis following a counter clockwise time course, and this hysteresis occurred both in the dark and the light period for both species. The hysteresis was not apparent when E_{CO_2} was plotted against T_{st} 12 min and 18 min earlier (prior temperature), for beech and oak, respectively (Figure 3.5b).

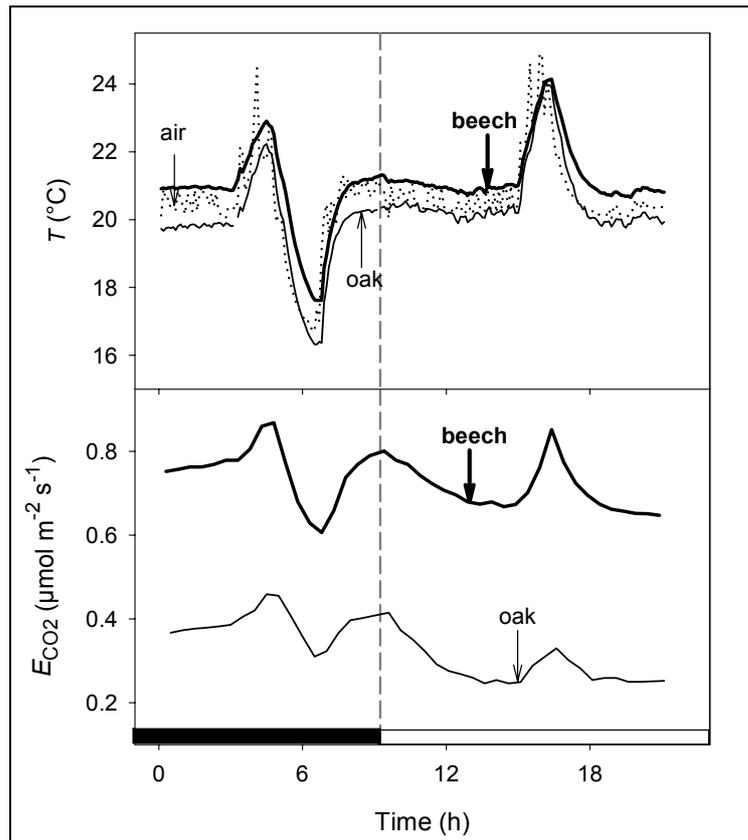


Figure 3.4: Time course of air and stem temperature (T) and stem CO_2 efflux rates (E_{CO_2}) for the beech and oak tree on day 99, when air temperature was altered stepwise. The vertical dashed line corresponds with the beginning of the light period.

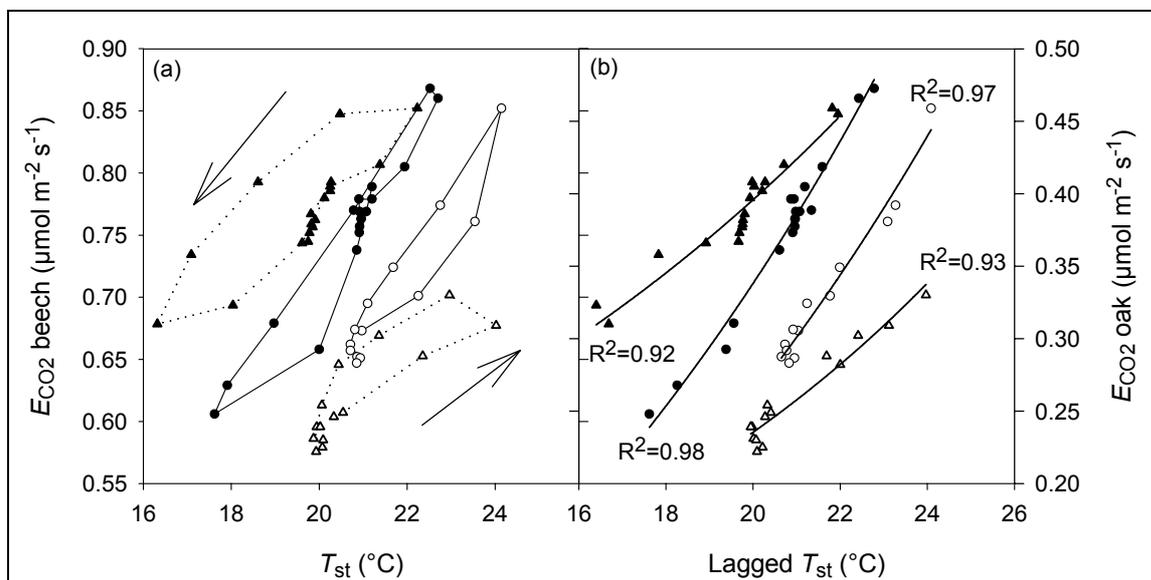


Figure 3.5: Stem CO_2 efflux rates (E_{CO_2}) from the beech segment (circles, solid lines) and oak segment (triangles, dotted lines) on day 99, shown separately for the light period (white symbols) and dark period (black symbols) versus (a) current stem temperature (T_{st}) and (b) lagged stem temperature. The time lag was 12 and 18 min for beech and oak, respectively. Coefficients of determination for the Q_{10} model (Equation 3.1) fit to the beech and oak data for the dark and light period are also given. Arrows show hysteresis.

Maintenance respiration rates of beech and oak were modelled for the dark and light period with Equation 3.1 using measured E_{CO_2} and lagged T_{st} . Table 3.1 summarizes estimated parameters and R^2 of the model. $E_{CO_2}(20)$ was lower for the light period than for the dark period, both for beech and oak. Q_{10} for the dark period was higher compared to the light period for beech, but lower for oak. For both species coefficients of determination were nearly equal for the dark and light period.

Table 3.1: Characteristics of the response of stem E_{CO_2} and xylem $[CO_2]$ to stem temperature. Q_{10} is the increase in respiration rate for a 10 °C increase in temperature, $E_{CO_2}(20)$ is the CO₂ efflux rate at 20 °C, $[CO_2](20)$ is the xylem CO₂ concentration at 20 °C (Equations 3.1 and 3.2). Standard errors of the mean are given in parentheses.

Measured variable	Lag period for best fit (min)	Q_{10}	$E_{CO_2}(20)$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$) or $[CO_2](20)$ (%)	R^2
E_{CO_2} beech dark	12	2.11 (0.065)	0.71 (0.0032)	0.98
E_{CO_2} beech light	12	2.06 (0.081)	0.62 (0.0053)	0.97
E_{CO_2} oak dark	18	1.98 (0.099)	0.40 (0.0026)	0.92
E_{CO_2} oak light	18	2.48 (0.170)	0.24 (0.0032)	0.93
$[CO_2]$ beech dark	0	1.52 (0.009)	2.43 (0.0021)	0.98
$[CO_2]$ beech light	0	1.45 (0.013)	2.43 (0.0044)	0.97

Xylem $[CO_2]$ of the beech stem also exhibited a strong relationship with T_{st} . The relationship shows a clear positive trend without a time lag (Figure 3.6), which can be well described by Equation 3.2. Parameters of the model are given in Table 3.1. Q_{10} is slightly higher for the dark period than for the light period, which is also the case for E_{CO_2} . $[CO_2](20)$ is the same for light and dark period.

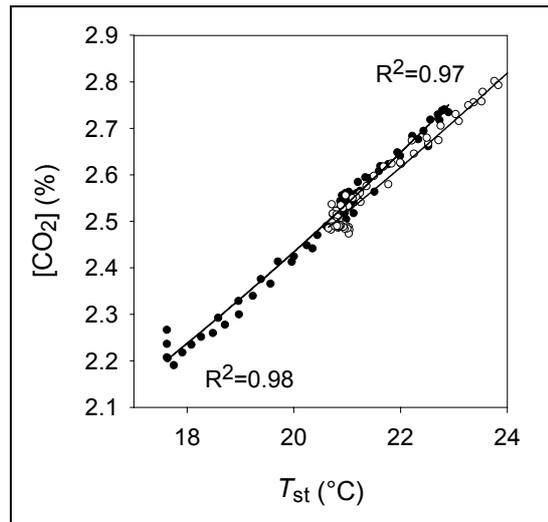


Figure 3.6: Xylem CO₂ concentration ($[CO_2]$) in the beech tree versus current stem temperature (T_{st}), shown separately for the dark period (●) and light period (○). Coefficients of determination for the Q_{10} model (Equation 3.2) fit to the data of the dark and light period are also given.

3.4 Discussion

3.4.1 Physiological variables

Stem E_{CO_2} was rather low, but was within the range of reported stem maintenance respiration rates. Damesin et al. (2002) reported values of 0.3 to 0.7 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at 15 °C for maintenance E_{CO_2} of *Fagus sylvatica* L. stems. Edwards and Hanson (1996) found maintenance respiration values between 0.1 and 0.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at 9.2 °C for two oak species with small diameters.

Stem $E_{\text{H}_2\text{O}}$ was very low compared to the values of leaf transpiration found in literature. It has been reported that permeability coefficients for peridermal water vapour diffusion resemble those of leaf cuticle (Schönherr and Ziegler 1980). Pilarski (1994) found that the diffusive resistance for H_2O of the bark in a 3-year-old shoot of *Syringa vulgaris* L. was 57 times greater in comparison with the stomatal resistance of the lower surface of a leaf. $E_{\text{H}_2\text{O}}$ depends on the species-specific bark structure: trees with smooth, dense periderms (e.g., *Fagus sp.*) transpire about one third of the amounts of species with fissured rhytidomal layers (e.g., *Quercus sp.*) (Pfanzen et al. 2002). In this study, stem $E_{\text{H}_2\text{O}}$ of oak was 4 times higher than that of beech.

Mean $[\text{CO}_2]$ measured in the xylem of the beech tree was 2.5%, which is in the range of reports using this and other techniques (Eklund 1990: ~ambient – 10%, Eklund 1993: 1 – 9%, Levy et al. 1999: 3 – 9.2%, McGuire and Teskey 2004: 0.7 – 18.7%). The internal CO_2 concentration in the xylem was 70 times higher than that of the ambient air, indicating that the resistance of the bark to lateral CO_2 diffusion was high. Evidence for restricted lateral gas movement in *Pinus strobus* branches has been given by Eklund and Lavigne (1995).

3.4.2 Temperature response of CO_2 efflux rate and xylem CO_2 concentration

Hysteresis between E_{CO_2} and T_{st} has been reported in several studies. Ryan et al. (1995) found that T_{st} lags up to 5 h provided the best fit to E_{CO_2} from stems of four conifers from 21 to 51 years old. Lavigne et al. (1996) found that the hysteresis between T_{st} and stem E_{CO_2} in balsam fir trees of 10 to 60 years old was not apparent when T data of 1.75 h earlier were used. Bosc et al. (2003) applied lag values up to 50 min to obtain a better fit between T_{st} and branch CO_2 efflux data in adult *Pinus pinaster* Ait. trees. Explanations for the hysteresis given in literature are: (1) measurements of T_{st} on one place are not representative for the T of the whole sapwood (Stockfors 2000), and (2) a delay exists between CO_2 production in the living tissues and CO_2 efflux at the organ surface because of the high resistance of the cambium and the bark (Eklund and Lavigne 1995). In

this study explanation (2) is likely to be the case, because no time lag was observed between xylem [CO₂] and T_{st} (Figure 3.6).

The relationship between lagged T_{st} and E_{CO_2} could be well described by the exponential model (Equation 3.1), both for the light and the dark period. A consistent exponential increase with increasing T has been reported by many authors (e.g., Levy and Jarvis 1998, Stockfors and Linder 1998). Q_{10} for the dark period compared to the light period was higher for the beech tree, but lower for the oak tree. Hence, there is no relationship between the Q_{10} value and the period of the day. The Q_{10} values for the young beech and oak tree ranged between 1.98 and 2.48, which are within the range of reported values for different broadleaved species. Ceschia et al. (2002) reported a mean Q_{10} of 2.04 for the upper stem part of *Fagus sylvatica* L. trees. Edwards and Hanson (1996) found a Q_{10} value of 2.4 for stems of *Quercus alba* L. and *Quercus prinus* L. species.

The response of xylem [CO₂] of the young beech tree to T_{st} could be described by the same exponential model as for E_{CO_2} . But in contrast with E_{CO_2} , xylem [CO₂] showed no delayed response to T_{st} (Figure 3.6). In most studies where [CO₂] has been measured *in situ*, it was also demonstrated that E_{CO_2} was well correlated with [CO₂] (McGuire and Teskey 2002, Teskey and McGuire 2002, McGuire and Teskey 2004) but in these studies E_{CO_2} and [CO₂] were not related to T_{st} .

3.4.3 Depressions in CO₂ efflux rate and xylem CO₂ concentration

Clear daytime depressions in stem E_{CO_2} were observed in both species. Daytime depressions in E_{CO_2} of stems and branches have been reported several times (e.g., Negisi 1978, Lavigne 1987, Kakubari 1988, Kaipainen et al. 1998). The following explanations have been proposed for this phenomenon: (1) respired CO₂ is transported with the transpiration stream (e.g., Martin et al. 1994), and (2) reduced cell water content in the stem causes a decrease in growth and maintenance processes and, hence, in respiration rate (e.g., Lavigne 1987, Wang et al. 2003). The first one is not of relevance in this study, because there was no sap flow at the time of measurement. Also the second explanation is not likely to be the cause of the depressions because there were no transpiring leaves at the time of measurement. Moreover, water losses from the stem (E_{H_2O}) were very low and did not significantly differ between the light and dark period. Also Ψ_{so} was high during the entire measurement period. And if drought stress would be the cause of the depressions, it is expected that Q_{10} values are lower during the light than during the dark. For example, Lavigne (1987) found that afternoon E_{CO_2} of young *Abies balsamea* (L.) Mill. trees was sometimes lower than morning E_{CO_2} and because Q_{10} values for the morning were significant higher than those for the

afternoon, he attributed this phenomenon to reduced stem metabolism in the afternoon. This was not the case in this study (Table 3.1).

How then can the significant daytime reductions in stem E_{CO_2} be explained? A reason that might have been overlooked is the influence of corticular and/or wood photosynthesis. Stem photosynthetic activity has been reported in a wide variety of plant species and life forms (e.g., Foote and Schaedle 1976a, Coe and McLaughlin 1980, Sprugel and Benecke 1991, Cernusak and Marshall 2000). Coe and McLaughlin (1980) measured winter season corticular photosynthesis in four deciduous tree species and concluded that corticular photosynthesis partially offsets maintenance costs of woody tissues during the leafless season. Damesin (2003) studied light responses of E_{CO_2} of current-year *Fagus sylvatica* L. stems and found substantial photosynthetic rates in the winter period. Foote and Schaedle (1976a) noted that corticular photosynthesis primarily uses internal CO_2 , i.e., it re-fixes CO_2 produced by woody tissue respiration. Wittmann et al. (2001) found that woody tissue respiration of young *Fagus sylvatica* L. trees was clearly reduced in the light because of the carbon re-fixation within the chlorenchymal tissues of twigs and stems. However, all measurements of corticular photosynthesis described in literature have been conducted with transparent cuvettes, in order to let the light reach the chlorophyll in the stem. In this study cuvettes were covered with several layers of aluminium foil and black tape, which prevented corticular and wood photosynthesis by the stem tissue enclosed in the cuvettes. Nevertheless, light was still able to reach the chlorophyll containing cells in the stem parts adjacent to the cuvettes and, hence, CO_2 was assimilated in those parts of the stem. It seems reasonable that during the light period a vertical internal CO_2 concentration gradient arises, with higher CO_2 concentrations in the stem segment covered by the cuvette and lower concentrations in the uncovered parts of the stem (Figure 3.7). According to Fick's law of diffusion, spontaneous transfer of molecules occurs from a region of high concentration to one of low concentration (Jones 1992). Hence, it seems reasonable that in our case axial CO_2 diffusion from the enclosed stem segment towards the uncovered stem parts occurred. Armstrong and Armstrong (2005) studied corticular photosynthesis in 3-year-old *Alnus glutinosa* (L.) Gaertn. trees and found that an increase in O_2 concentration in the stem as a result of corticular photosynthesis enhanced O_2 diffusion towards the roots. Hence, they demonstrated that corticular photosynthesis is able to affect stem internal diffusive gas transport.

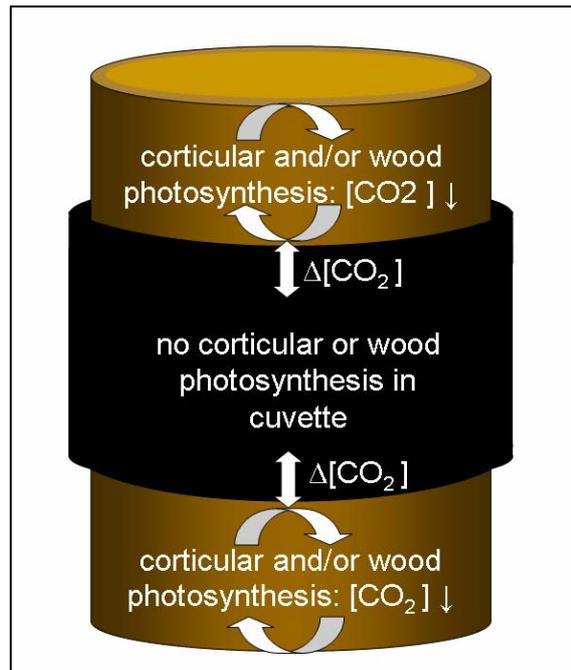


Figure 3.7: Schematic of the hypothesized CO₂ fluxes in a stem segment as a consequence of corticular and/or wood photosynthesis. Due to photosynthesis, the CO₂ concentration ([CO₂]) decreases in the stem tissue adjacent to the opaque cuvette (black cylinder) and an axial [CO₂] gradient arises between the tissues inside and outside the cuvette.

In our study, the influence of the internal CO₂ concentration in the stem parts adjacent to the cuvette on E_{CO_2} of the enclosed stem segment has also been observed when a hole for the insertion of a Teflon tube was drilled in the wood 1 cm below the stem cuvette. After the drilling, a sharp decrease in E_{CO_2} of the stem segment was observed. After 3 hours E_{CO_2} reached a minimum value of $0.02 \mu\text{mol m}^{-2} \text{s}^{-1}$ and then slowly recovered, reaching the original rate after 5 days. This indicates that drilling a hole and, hence, removing a barrier for CO₂ diffusion (Teskey and McGuire 2005) has a substantial effect on E_{CO_2} at places in the neighbourhood of this hole. Escape of CO₂ through the hole causes a CO₂ gradient along the stem, which in turn, causes axial diffusion of CO₂ molecules from the parts with a high [CO₂] towards the parts with a low [CO₂]. A reduction in the radial CO₂ diffusion from the stem towards the atmosphere at places in the neighbourhood of the hole is then a logical result. However, more extensive research on woody tissue E_{CO_2} during the dormant season is necessary, and further experiments to provide evidence on the possible role of corticular and/or wood photosynthesis in daily variations of stem E_{CO_2} remain to be conducted.

3.5 Conclusions

Stem E_{CO_2} of young leafless trees exhibited significant lower values during the light period, despite constant T_{st} in the light and dark period. Measurement of xylem [CO₂] confirmed that depressions in E_{CO_2} actually corresponded with a

reduction of the internal CO₂ concentration. Variations in E_{CO_2} that were not related to T have previously been reported and explanations for this phenomenon were (1) transport of respired CO₂ with the sap flow or (2) reduced respiration due to a lower cell water content during the day. In our case the lower $E_{CO_2}(20)$ during the light period could neither be explained by sap flow transporting CO₂, since no sap flow occurred at the time of measurement, nor could it be explained by reduced respiration because of low cell water content, since there was no significant difference in stem E_{H_2O} between the dark and light period and Q_{10} of stem E_{CO_2} for the light period was not consistently lower than Q_{10} for the dark period. Hence, previously formulated theories for daytime depressions in E_{CO_2} failed to explain our results. This indicates that other mechanisms have to be involved in temperature-independent variations in stem E_{CO_2} . A plausible explanation is that re-fixation of respired CO₂ by corticular and/or wood photosynthesis in the stem parts adjacent to the stem segment enclosed by the cuvette might have caused the observed daytime depressions. However, the potential role of corticular and wood photosynthesis in daily variations of stem E_{CO_2} needs to be further explored.

Chapter 4

Daytime depression in tree stem CO₂ efflux rates: is it caused by low stem turgor pressure?

Adapted from : Saveyn A, Steppe K and Lemeur R (2007) Daytime depression in tree stem CO₂ efflux rates: is it caused by low stem turgor pressure? *Annals of Botany* 99: 477 – 485

Abstract

Daytime CO₂ efflux rates (E_{CO_2}) from tree stems are often reported to be lower than expected from the exponential relationship between temperature and respiration. Explanations of daytime depression in E_{CO_2} have focused on the possible role of internal CO₂ transport in the xylem. However, another possible cause that has been overlooked is the daily dynamics of the water status in the living stem tissues and its influence on stem growth rate and thus respiration. The objective of this study was to assess the daily dynamics of stem water status and growth rate and to determine the extent to which they may be linked to daily variations in stem E_{CO_2} . E_{CO_2} of young beech and oak stems was measured under controlled conditions. Relative turgor pressure (Ψ_p) in the external living stem tissues, obtained from simulations with the RCGro model, was used as an indicator of the water status in the living stem tissues. Daily dynamics of stem growth were derived from Ψ_p : growth was assumed to occur when Ψ_p exceeded a relative threshold value. There was a strong correspondence between fluctuations in E_{CO_2} and simulated Ψ_p . The non-growth-conditions during daytime coincided with depressions in E_{CO_2} . Moreover, E_{CO_2} responded to changes in Ψ_p in absence of growth, indicating that also maintenance processes were influenced by the water status in the living stem tissues. It is suggested that water status of tree stems is a potential important determinant of stem E_{CO_2} as it influences the rate of growth and maintenance processes in the living tissues of the stem.

4.1 Introduction

Respiration rates of woody tissues are commonly measured by enclosing the tissue in a cuvette, and measuring the rate of CO₂ efflux from the tissue with an infrared gas analyzer (IRGA). It is assumed that all the CO₂ respired by the woody tissue enclosed in the cuvette diffuses radially from the stem interior into the cuvette. Several studies (e.g., Negisi 1972, 1975, 1978, 1981, Lavigne 1987, Kakubari 1988, Kaipiainen et al. 1998, Gansert and Burgdorf 2005) showed that on warm sunny days, measured stem CO₂ efflux rate (E_{CO_2}) was lower than that expected from the exponential relation between respiration and temperature (Amthor 1989):

$$R_S = R_S(T_r) \times Q_{10}^{\frac{T-T_r}{10}} \quad (4.1)$$

with R_S the respiration rate at temperature T , $R_S(T_r)$ the respiration rate at a reference temperature T_r and Q_{10} the relative increase in respiration rate with a 10 °C rise in temperature. This phenomenon is the so-called daytime depression in stem E_{CO_2} . Several authors suggested that sap flow rate (f_s) might have an influence on stem E_{CO_2} (Negisi 1979, Ryan 1990, Sprugel 1990, Hari et al. 1991, Martin et al. 1994, McGuire and Teskey 2004, Bowman et al. 2005). This explanation is based on the high solubility of CO₂ in water, so that a portion of the CO₂ evolved by the respiring cells of woody tissues might dissolve in the sap and be transported vertically in the xylem along with the transpiration stream instead of moving radially through the stem into the cuvette (Hari et al. 1991, Kaipiainen et al. 1998). Hence, measured values of stem CO₂ efflux may be affected by the rate of CO₂ diffusion in xylem sap rather than by respiratory processes per se.

However, transport of dissolved CO₂ in the xylem is only one consequence of sap flow. According to the cohesion tension theory, water in the xylem of transpiring trees is under considerable tension as it is pulled from soil to leaves along a complex pathway of water conducting elements which together have a large hydraulic resistance (Irvine and Grace 1997). When tension develops in the xylem, a water potential gradient develops between the phloem and xylem, which leads to a water flux from the phloem across the cambium towards the xylem (Garnier and Berger 1986). Hence, water reserves of the living tissues external to the xylem are depleted during the daytime, resulting in stem diameter shrinkage. These living tissues respire to provide energy for growth and maintenance processes. When the water reserves in the living tissues external to the xylem are depleted, water deficits may occur, temporarily reducing rates of growth and maintenance processes and the respiratory processes which support them (Lavigne 1987, Kakubari 1988, Wang et al. 2003, Daudet et al. 2005). Particularly expansion growth may be reduced by water deficit: it is one of the most sensitive

of all plant processes to drought stress (Hsiao 1973). However, it is difficult to actually determine the occurrence of water deficit in the stem tissue. Wang et al. (2003) measured xylem water potential of *Larix gmelini* Rupr. branches, which was greater (less negative) in the morning than afternoon, but this is not evidence that growth and/or maintenance processes in the living tissues are actually suppressed. It is not the xylem water potential, but the turgor pressure (Ψ_p) in the stem tissue that reflects the water status in the living cells of the stem (phloem, cambium and parenchyma) (Bradford and Hsiao 1982). Growth processes, such as cell formation, cell wall expansion and deposition of new wall material are more dependent on Ψ_p and cell volume than on water potential (Boyer 1968, Hsiao et al. 1976, Ray 1987, Proseus and Boyer 2006). The cell turgor is in direct proportion to the water potential only if the osmotic potential of the cell remains constant. However, turgor and cell volume can be maintained by osmotic adjustment (i.e., the active accumulation of solutes in the symplast), which may serve to sustain growth (Woodruff et al. 2004). Hence, it is the daily course of Ψ_p that can reveal when cell growth is likely to occur, and not the daily course of the water potential. A widely used model of cell expansion, developed by Lockhart (1965), relates relative cell expansion to cell Ψ_p , cell wall extensibility (Φ) and a threshold Ψ_p at which wall yielding occurs (Γ):

$$\text{relative cell expansion} = \Phi \times (\Psi_p - \Gamma) \quad (4.2)$$

Ψ_p must be above this threshold value for the cell to expand irreversibly. This idea has been incorporated into a mathematical model linking sap flow dynamics in trees to daily fluctuations in stem diameter and radial growth (Steppe et al. 2006). This model, which requires only transpiration rate of the whole tree as input variable, simulates the change of Ψ_p relative to the maximum Ψ_p , occurring at zero f_s .

In order to explain daytime depressions in E_{CO_2} , previous studies have examined the link between f_s and stem E_{CO_2} to test the hypothesis that sap flow directly determines internal CO₂ transport in the xylem. However, sap flow also determines the course of Ψ_p in the living stem tissues, which as discussed is a vital driving variable for cell growth and, consequently, for the associated energy demand. We know of no studies that have attempted to evaluate the effects of Ψ_p and cell growth on the daily course of stem E_{CO_2} . Therefore, the objective of this study was to assess the daily dynamics of Ψ_p and growth, and to determine their association with daytime depressions in stem E_{CO_2} . The present work does not aim at refuting previous explanations for daytime depressions in stem E_{CO_2} , but introduces strong, if circumstantial evidence for the role of Ψ_p and the daily dynamics of cell growth as a cause of daytime depressions in stem E_{CO_2} .

4.2 Materials and methods

4.2.1 Plant material and growth chamber

The experimental setup was very similar as in the previous study (Figure 3.1). Two different tree species, a ring-porous oak (*Quercus robur* L.) and a diffuse-porous beech (*Fagus sylvatica* L.), were studied in order to examine the possible link between daytime depressions of E_{CO_2} and Ψ_p . A 3-year-old beech tree, previously grown outdoors was planted at the beginning of February 2004 in a 50 l container, filled with potting mixture. The tree was 1.55 m high and the stem diameter at the soil surface was 16.4 mm. A 3-year-old oak tree was studied in 2005; it was 1.6 m high and had a stem diameter at the soil surface of 19.2 mm. The trees were placed in a growth chamber with dimensions 2 x 1.5 x 2 m (height x width x length), to control radiation and air temperature. Light was from densely packed fluorescent lamps ('TL'D 80, Philips Lighting NV, Brussels, Belgium), producing a photon flux (400-700 nm) of photosynthetic active radiation (PAR) of approximately $470 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the top of the trees (measured with a quantum sensor, Model Li-190S, Li-COR, Lincoln, TE, USA). The trees were watered weekly and fertilized monthly with a NPK plus micronutrient mix (Substral, Sint-Niklaas, Belgium). Measurements were performed in the beginning of the growing season of 2004 for the beech tree and 2005 for the oak tree. During the measurements, the leaf area of the trees rapidly increased.

On one day during the experiment (day 154), from 1400 h until 1800 h, the leaves of the oak tree were enclosed in large transparent plastic bags in order to decrease the transpiration rate, while the light and temperature in the chamber were kept constant.

4.2.2 CO₂ efflux measurement

Gas exchange was measured on a 13-cm long segment of each stem, approximately 0.7 m above soil surface. The lower and upper diameter of the segments were 8.3 and 8.0 mm, respectively for the beech, and 11.0 and 10.7 mm, respectively for the oak stem. The segments were enclosed in air-tight cylindrical acrylic cuvettes with a diameter of 6 cm covered with aluminium foil and black tape. The cuvettes and the stem parts above and below the cuvettes were covered with reflective bubble insulation, which prevented all light from reaching the tissue in order to avoid cortical and wood photosynthesis. To minimize short-term fluctuations in CO₂ concentration within the stem cuvettes, air from the growth chamber was pumped by a membrane pump (Model N 86.KN 18, KNF Verder, Aartselaar, Belgium) at a flow rate of 1 l min^{-1} into a 50 l buffer tank before entering the cuvettes. Air leaving the cuvettes was first partially dried at 4 °C with a gas cooler (CG/G 73-4, Hartmann and Braun AG, Germany), before the CO₂ concentration was measured with an infrared gas analyzer

(IRGA; Binos 100-4P, Fisher-Rosemount, Hasselroth, Germany), as the difference between the air leaving the stem cuvette and a reference cuvette, which did not contain a stem segment. The system was automatically zeroed every 450 s and 360 s, for the beech and oak tree respectively, by passing air from the reference cuvette through the reference and measuring cells of the IRGA. Since only living cells are producing CO₂ and the largest fraction of living cells in young trees is located close to the surface (phloem and cambium) (Stockfors and Linder 1998), CO₂ efflux rates (E_{CO_2}) were expressed per unit of stem surface area.

4.2.3 Stem diameter measurement

Stem diameter (D) was measured using a linear variable displacement transducer (LVDT) and transducer bridge (respectively LBB, 375-PA-100 and 8C-35, Schaevitz, Hampton, VA, USA), placed 1 cm below the cuvette. The LVDT was supported by a stainless steel holder; tests with a 12-mm diameter aluminium rod showed that no temperature correction was required.

4.2.4 Sap flow measurement

Sap flow rates (f_s) at the stem base, and on a second order branch (at the tree top), were measured with flow sensors based on the heat balance principle (Models SGB16 and SGA5, Dynamax Inc., Houston, TX, USA), and installed according to the operation manual, as was calculation of f_s (van Bavel and van Bavel 1990). The sensors were thermally insulated with several layers of aluminium foil. Sheath conductance of the gauge was recalculated daily using minimum values in darkness between 0400 h and 0700 h. The value for thermal conductance of woody stems of $0.42 \text{ W m}^{-1} \text{ }^\circ\text{C}^{-1}$ was taken from Steinberg et al. (1989).

4.2.5 Data acquisition

All signals from sensors and devices were logged (HP 34970A, Hewlett Packard, Palo Alto, CA, USA) at 10-s intervals, and monitored continuously (Hewlett-Packard programme VEE). All sensor signals were averaged over 450-s and 360-s periods, for the beech and oak tree respectively, and recorded by a computer.

4.2.6 Temperature correction for CO₂ efflux rates

Air temperature (T_a) and stem temperature (T_{st}) were measured with copper-constantan thermocouples (Omega, Amstelveen, Netherlands). For T_{st} a 1-mm diameter hole, 7 mm deep was drilled in the stems into which the thermocouple was inserted. Although a constant temperature was set for the growth chamber,

there were small variations, so the measured E_{CO_2} was adjusted to T_{st} of 20 °C using the following equation (based on Equation 4.1):

$$E_{\text{CO}_2}(20) = E_{\text{CO}_2} / Q_{10}^{\frac{T_{\text{st}} - 20}{10}} \quad (4.3)$$

with $E_{\text{CO}_2}(20)$ the calculated CO₂ efflux rate at 20 °C (expressed in $\mu\text{mol m}^{-2} \text{s}^{-1}$). Q_{10} was estimated by ordinary least squares, based on measurements of E_{CO_2} on days where T_a was altered stepwise (four temperature steps of 1 h (24 – 21 – 17 – 21 °C)) during the dark period of day 121 for beech and of day 148 for oak. Calculation of Q_{10} was based on E_{CO_2} data of the dark period, since then no sap was flowing and it was assumed that the stem tissue was fully hydrated.

4.2.7 Simulation of stem turgor pressure

Non-destructive measurement of Ψ_p is difficult, so the mechanistic flow and storage model RCGro, developed by Steppe et al. (2006), was applied to simulate the change of stem Ψ_p relative to the maximum Ψ_p , at zero f_s . The model (Figure 4.1) enables simulation of tree f_s dynamics (water transport submodel) which are directly linked to variations in D (stem diameter variation submodel), using the radial flow of water (Figure 4.1, bold black arrow) between the xylem (considered as a continuous rigid cylinder) and the stem storage compartment (i.e., the living extensible cells external to the xylem). This radial flow causes changes in the water content of the external living tissues and, hence, in Ψ_p of these tissues. D varies due to reversible stem shrinkage/swelling and irreversible radial stem growth. If Ψ_p is smaller than the threshold Ψ_p at which wall yielding occurs (Γ), then variations in D only reflect reversible shrinkage/swelling. If Ψ_p is larger than Γ , then irreversible radial growth occurs, in addition to shrinkage/swelling (Figure 4.1). For a detailed description of the model see Steppe et al. (2006). Whole-tree transpiration rate, which is the input variable for the model, is normally determined by multiplying measured branch f_s (not stem f_s since stem f_s lags behind transpiration) by the ratio of total leaf area/leaf area upstream of the sap flow sensor (Steppe et al. 2006). However, since the leaf area of both trees rapidly increased, it would have been necessary to measure total leaf area every day, which is a very laborious task. Therefore, f_s measured at the top of each tree was increased each day by multiplying by a scaling factor, the ratio between the daily sum of stem f_s and branch f_s . This upscaled branch f_s was used as the input variable for the model. Stem f_s and variations in D were selected as suitable variables for model calibration, using the simplex method (Nelder and Mead 1965) by minimizing the sum of squared errors between the simulated values and the measured data of stem f_s and variations in D (Steppe et al. 2006). The model was calibrated for beech and oak separately, and allowed us to simulate relative changes in stem Ψ_p throughout the day, as an indicator of the water status in the living tissues of the stem.

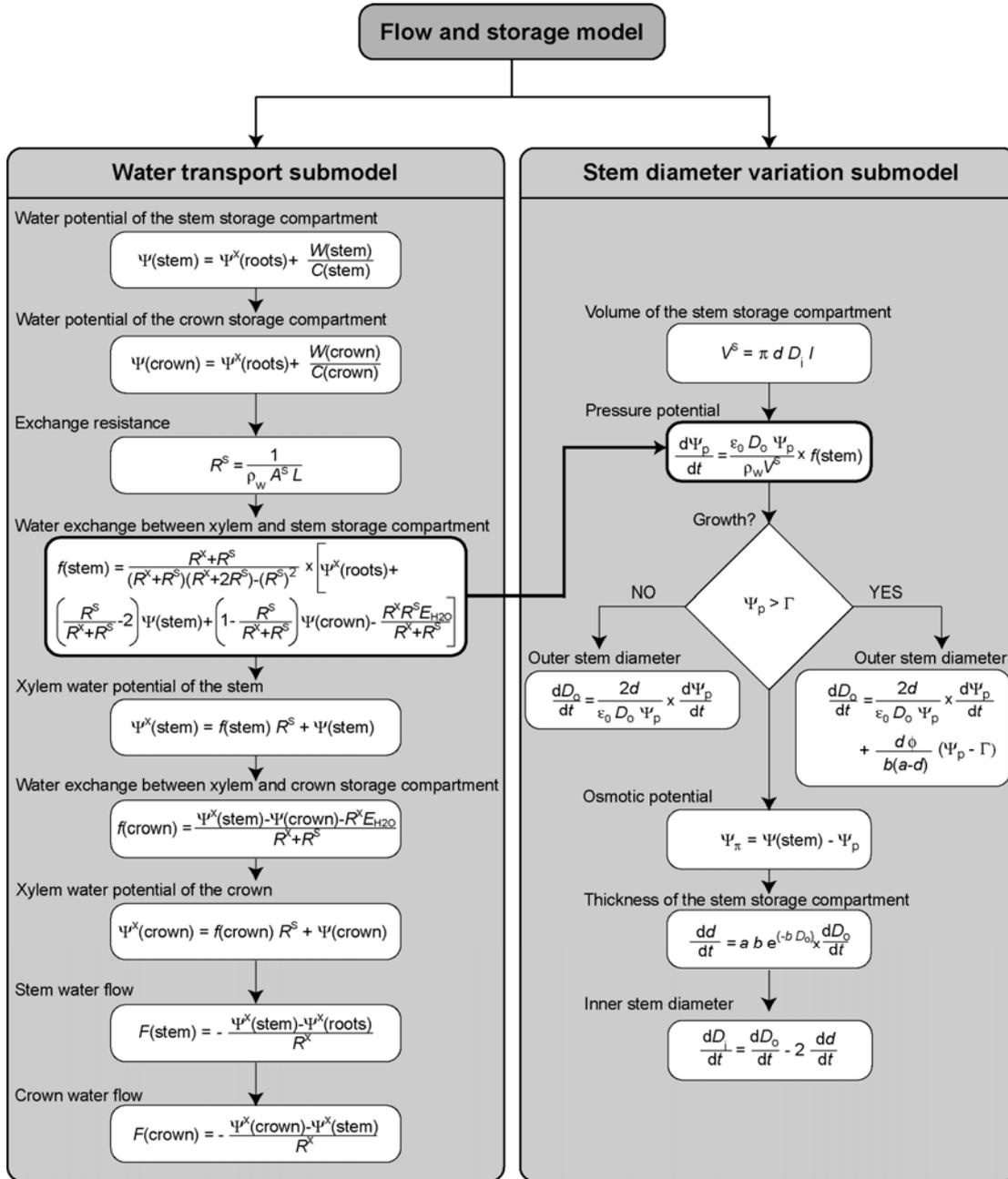


Figure 4.1: Schematic of the model linking the dynamics of tree sap flow and storage to changes in stem diameter and growth. Ψ = total water potential of storage compartment, Ψ^x = total water potential of xylem compartment, W = water content of storage compartment, C = capacitance of storage compartment, R^s = flow resistance between xylem and storage compartment, R^x = flow resistance in xylem compartment, ρ_w = density of water, A^s = surface area of the virtual membrane separating the stem storage compartment from the xylem compartment, L = hydraulic conductivity of the membrane, f = sap flow between xylem and storage compartment, E_{H_2O} = transpiration, F = sap flow in a xylem compartment, V^s = volume of storage compartment, d = thickness of storage compartment, D_i = inner diameter of stem segment, D_o = outer diameter of stem segment, l = length of stem segment, Ψ_p = turgor pressure potential, ε_0 = proportionality constant, Γ = threshold Ψ_p at which wall yielding occurs, ϕ = cell wall extensibility, a and b = allometric parameters, Ψ_π = osmotic potential.

Simulated relative Ψ_p was continuously compared with the relative threshold value Γ (i.e., expressed as a percentage of maximum Ψ_p occurring at zero f_s) in order to estimate during which periods of the day irreversible radial stem growth occurred (Equation 4.2).

4.3 Results

4.3.1 Daytime depression in CO_2 efflux rate

Despite the higher T_{st} during the day than night, daytime E_{CO_2} of both tree stems was lower compared to night (Figure 4.2). When E_{CO_2} was adjusted for variations in T_{st} using Equation 4.3 ($E_{\text{CO}_2(20)}$), with estimated Q_{10} values of 3.2 (SE = 0.20) and 2.0 (SE = 0.17) for beech and oak, respectively, the daytime depressions in E_{CO_2} became even more pronounced (Figure 4.2).

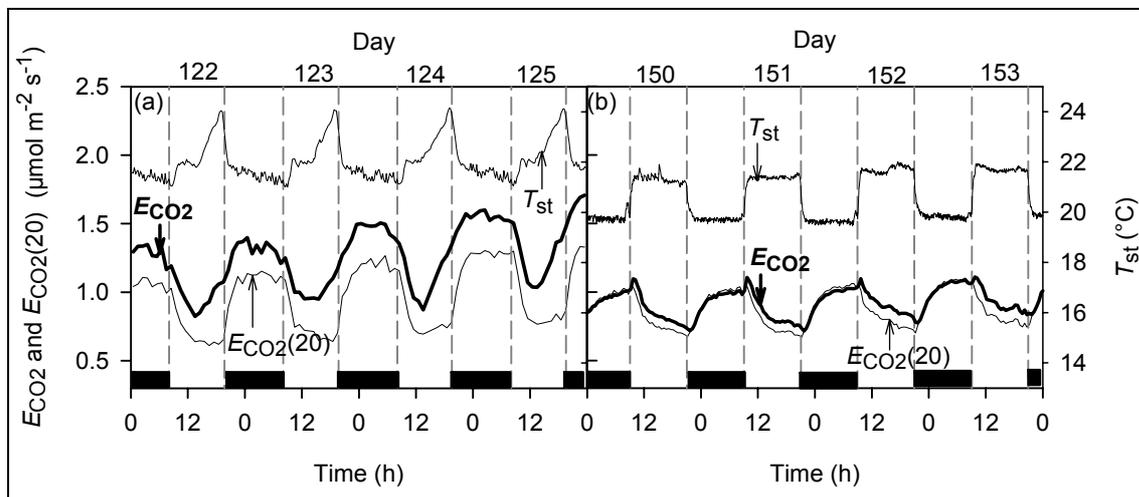


Figure 4.2: Daily patterns of measured temperature (T_{st}) and CO_2 efflux rate (E_{CO_2}), and of CO_2 efflux rate normalized to 20 °C ($E_{\text{CO}_2(20)}$) for stems of (a) beech (days 122 – 125) and (b) oak (days 150 – 153). Beginning and end of each dark period are indicated by black boxes and vertical dashed lines. Values of $E_{\text{CO}_2(20)}$ were obtained with Equation 4.3, using estimated Q_{10} values of 3.2 and 2.0 for beech and oak, respectively.

When the leaves of the oak tree were enclosed in plastic bags during 4 hours on day 154, transpiration decreased substantially, although temperature and light conditions were not altered. In that case an increment in stem $E_{\text{CO}_2(20)}$ was observed (Figure 4.3). Hence, factors other than temperature variations appeared to exert a control over stem E_{CO_2} .

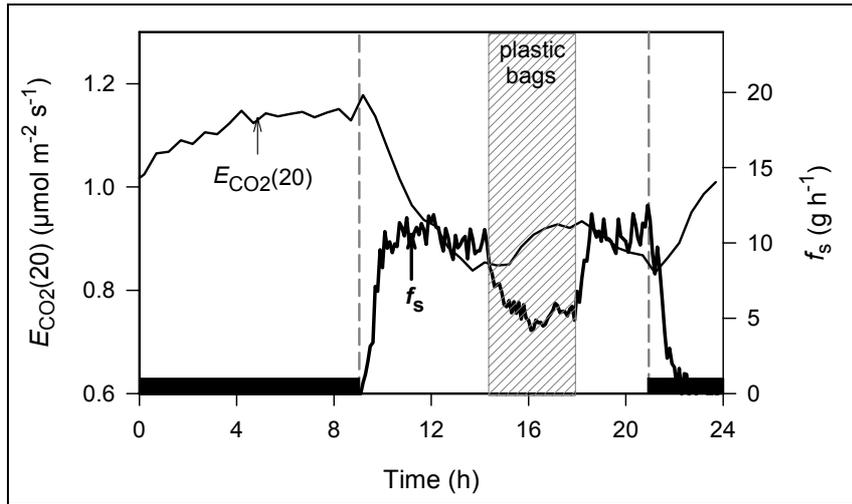


Figure 4.3: Time course of CO₂ efflux rate from stems, normalized to 20 °C ($E_{\text{CO}_2(20)}$), obtained with Equation 4.3 ($Q_{10} = 2.0$), and sap flow rate at stem base (f_s) for the oak tree on day 154, when the leaves were enclosed in transparent plastic bags from 1400 h until 1800 h. Black boxes indicate the dark periods.

4.3.2 Model input and outputs

The RCGro model used the upscaled branch f_s as the only input variable. The values of the estimated model parameters (Table 4.1) were similar to those obtained by Steppe (2004) and Steppe et al. (2006, 2007) for young beech and oak trees. Figure 4.4 shows how well the output variables D and stem f_s of the RCGro model fit the measured values for both trees. Coefficients of determination (R^2) of the linear regression between measured and simulated data were high for both trees.

Table 4.1: Values of the estimated model parameters for beech and oak. R^x = flow resistance in xylem compartment, $C(\text{stem})$ = capacitance of stem storage compartment, $C(\text{crown})$ = capacitance of crown storage compartment, Φ = cell wall extensibility, Γ = threshold Ψ_p at which wall yielding occurs.

Parameter	Beech	Oak
R^x (MPa s mg ⁻¹)	0.1478	0.1701
$C(\text{stem})$ (mg MPa ⁻¹)	638.6	1158.4
$C(\text{crown})$ (mg MPa ⁻¹)	350.3	5063.4
Φ (MPa ⁻¹ s ⁻¹)	6.95×10^{-7}	5.16×10^{-7}
Relative Γ (% of full turgor)	92.86	95.66

4.3.3 Stem turgor pressure versus CO₂ efflux rate normalized to 20 °C

Changes in Ψ_p in the external living stem tissues, simulated with the calibrated RCGro model, and $E_{\text{CO}_2(20)}$ during the period day 122 – 125 for beech and 150 – 154 for oak are shown in Figure 4.5. At the onset of each light period, Ψ_p decreased sharply, due to the depletion of the water reserves in the stem tissues.

This is also observed in the decrease of diameter (D ; Figure 4.4c and d). During the light periods, Ψ_p became lower than the critical wall-yielding threshold value needed for irreversible radial stem growth to occur. At the beginning of each dark period, Ψ_p increased sharply due to the replenishment of the water reserves in the stem. The replenishment is also observed as an increase of D (Figure 4.4c and d). During darkness, Ψ_p exceeded the wall-yielding threshold value. The daily variations of Ψ_p corresponded closely with the temperature-independent variations of E_{CO_2} . Upon enclosure of the oak foliage in the plastic bags on day 154, Ψ_p in the stem increased but remained lower than the wall-yielding threshold value necessary for irreversible growth (Figure 4.5b). Nevertheless, $E_{CO_2}(20)$ increased.

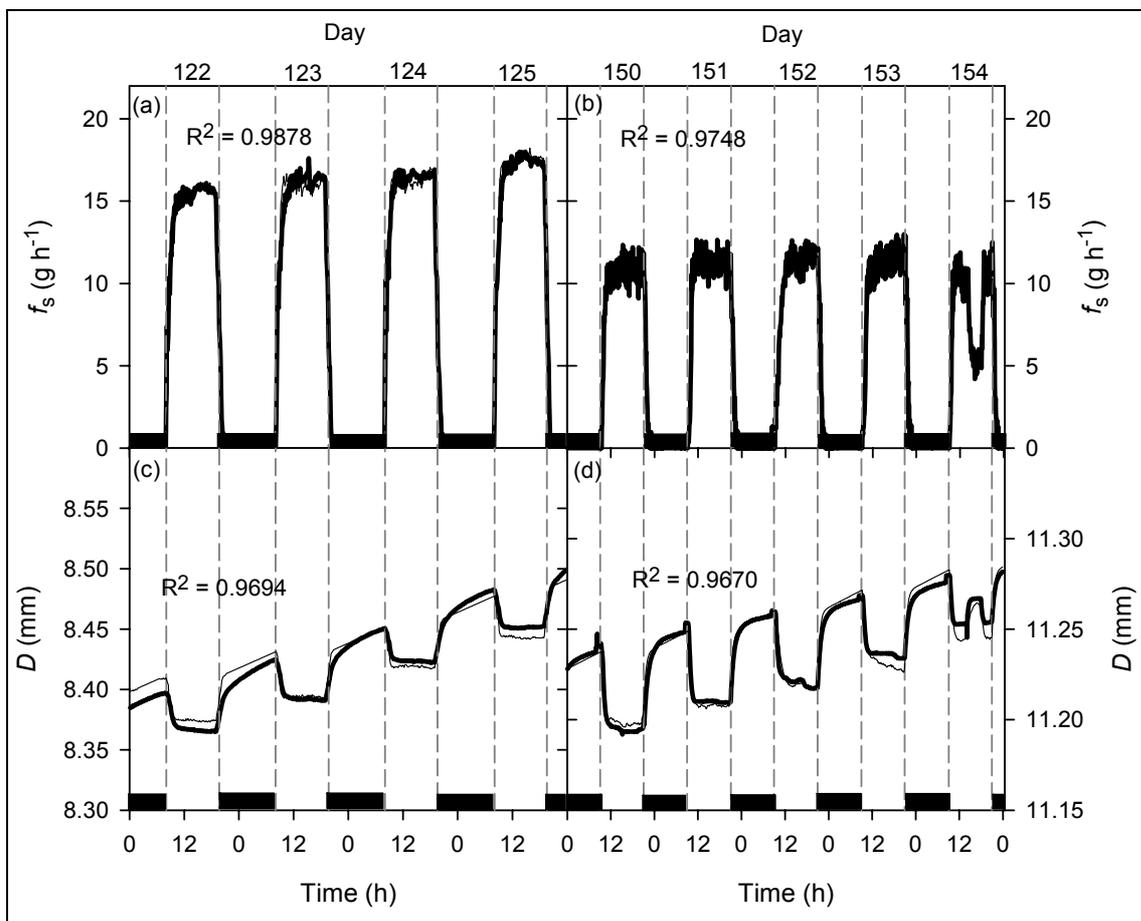


Figure 4.4: Comparison between measured (thick lines) and simulated (thin lines) sap flow rates (f_s) at the stem base for beech (a) and for oak (b), and between measured (thick lines) and simulated (thin lines) stem diameter (D) for beech (c) and oak (d). Beginning and end of each dark period are indicated by black boxes and vertical dashed lines. The coefficients of determination (R^2) of the linear regression between measured and simulated values are also given.

Closer inspection of the data of $E_{CO_2}(20)$ and Ψ_p (Figure 4.6a and b), showed that $E_{CO_2}(20)$ lagged behind Ψ_p , and hysteresis, following a counter-clockwise time

course, is clearly visible when $E_{\text{CO}_2(20)}$ is plotted as a function of Ψ_p (Figure 4.6c and d).

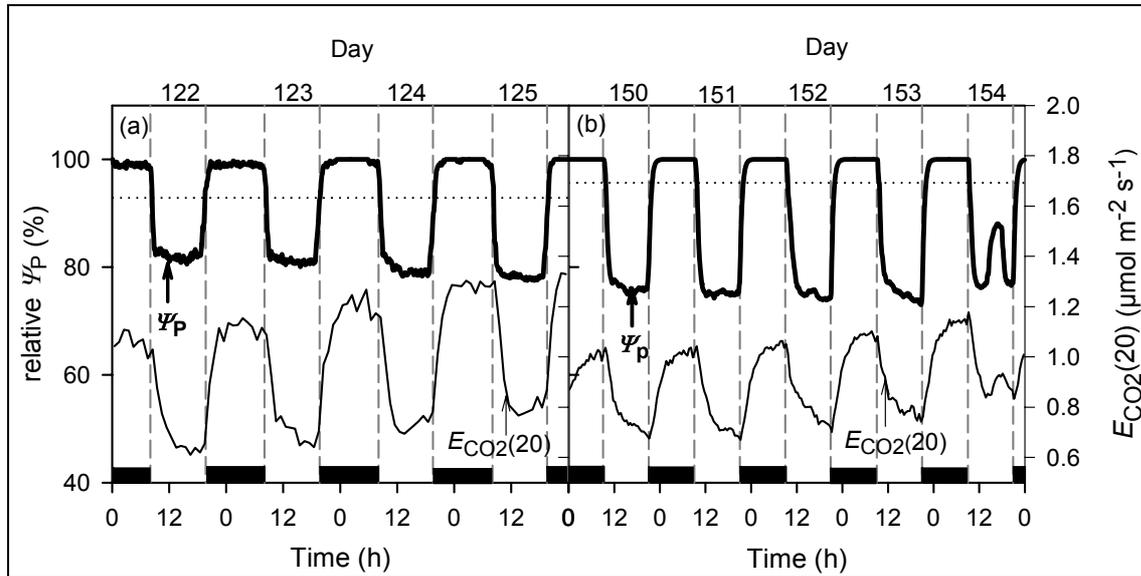


Figure 4.5: Simulated turgor pressure (Ψ_p) in the external living stem tissues, expressed as a percentage of maximum Ψ_p occurring at zero f_s , and calculated CO₂ efflux rate normalized to 20 °C ($E_{\text{CO}_2(20)}$) during the period days 122 – 125 for beech (a) and 150 – 154 for oak (b). Ψ_p was simulated with the calibrated RCGro model. Values of $E_{\text{CO}_2(20)}$ were calculated with Equation 4.3, using a Q_{10} of 3.2 and 2.0 for beech and oak, respectively. The horizontal dotted line represents the relative wall-yielding threshold value for Ψ_p above which radial stem growth occurs. Start and end of each dark period are indicated by black boxes and vertical dashed lines.

4.4 Discussion

We observed clear daytime depressions in stem E_{CO_2} , compared to the night, despite a higher T_{st} , suggesting that factors other than temperature controlled E_{CO_2} (Figure 4.1). The experiments were conducted during spring, when the tree growth rate was high, and growth respiration is the dominant contributor to total stem respiration (e.g., Stockfors and Linder 1998). Growth processes are very sensitive to drought stress (Hsiao 1973), and so very probably depend on the dynamics of the water status in the living stem tissues. This study used a simulation model approach to estimate Ψ_p , which is a good indicator of the water status of the living tissues. Ψ_p fluctuated daily due to the dynamics (depletion and replenishment) of water reserves in the stem. As a result, relative radial stem growth rate fluctuated daily, with the highest growth rate occurring during the night when Ψ_p exceeded the wall-yielding threshold value (Γ) necessary for cell growth (Figure 4.5). Simulated and measured stem diameter patterns both illustrate that irreversible radial stem growth occurred during the night: D increased continuously (Figure 4.4c and d). Differences in plant growth rate during day and night have been studied by several authors. Boyer (1968)

observed that enlargement of leaves of a well-watered sunflower plant (*Helianthus annuus* L.) was 5 to 6 times higher at night than during the day. Also Schurr et al. (2000) found that leaf growth rates in *Ricinus communis* L. peaked during the late night and were minimal in the late afternoon. Halter et al. (1996) observed that root elongation rates in *Eucalyptus nitens* (Deane and Maiden) Maiden and *E. pauciflora* Sieber ex Sprengel seedlings were 60 and 67% higher, respectively, during the night than during the day. In our study, daytime Ψ_p was lower than the wall-yielding threshold value which must be exceeded for expansion growth (Figure 4.5), indicating that the stem growth rate of both trees was zero during the daytime. Measurements of stem diameter confirm this: D was constant during the day (Figure 4.4c and d). Hence, the higher $E_{CO_2(20)}$ during darkness compared to the light can be explained at least partially by the higher energy demand to support growth in the stem tissue.

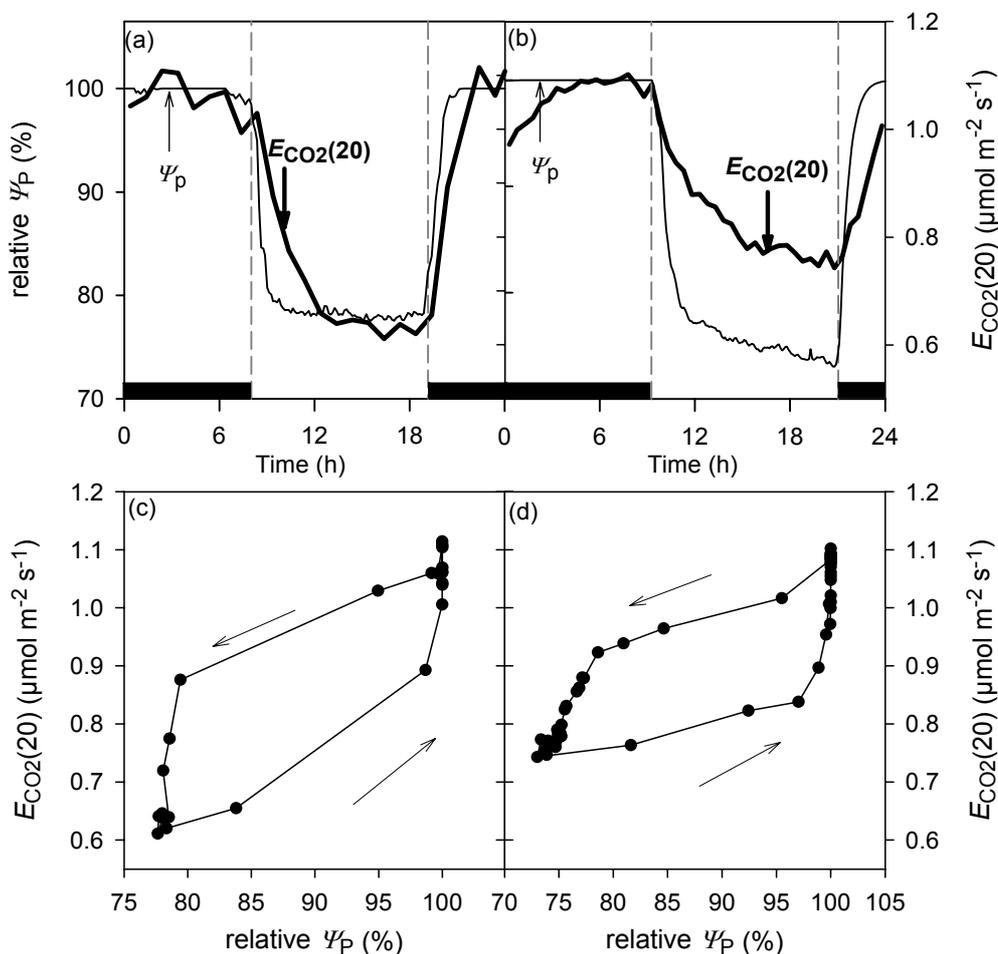


Figure 4.6: Time course of simulated stem turgor pressure (Ψ_p), expressed as a percentage of maximum Ψ_p occurring at zero f_s , and CO₂ efflux rate normalized to 20 °C ($E_{CO_2(20)}$) on day 122 for beech (a) and 153 for oak (b). Values of $E_{CO_2(20)}$ were calculated with Equation 4.3, using a Q_{10} of 3.2 and 2.0 for beech and oak, respectively. Black boxes indicate the dark periods. Hysteresis occurs between Ψ_p and $E_{CO_2(20)}$ for beech (c) and oak (d). Measurement symbols are connected chronologically. Arrows show hysteresis.

When transpiration of the oak tree was decreased by enclosure (Figure 4.3 and 4.5b), Ψ_p increased but did not exceed the wall-yielding threshold value. Hence, cell growth was not expected to occur during this period. Nevertheless, $E_{CO_2}(20)$ increased. This indicates that E_{CO_2} increased with any improvement of the water status in the living stem tissues, even when stem expansion growth did not yet take place. A plausible explanation is that with any increase in Ψ_p and, hence, an improved water status, the rate of maintenance metabolism (such as protein turnover, membrane repair, etc.) increased. In contrast to growth, maintenance metabolism always occurs, even under stress conditions. However, when conditions become more favourable (e.g., an enhanced water status in the living stem tissues), the rate of maintenance metabolism and, hence, E_{CO_2} will be enhanced. Unfortunately, we are unaware of any studies on the relationship between turgor and rates of maintenance processes. It is also important to note that cell wall expansion is not the only growth process in stems. Growth also includes processes such as cell wall deposition and assembly. Proseus and Boyer (2006) recently demonstrated that by decreasing Ψ_p , wall deposition and assembly in *Chara corallina* cells decreased. Hence, not only wall expansion is affected by changes in Ψ_p . However, Proseus and Boyer (2006) did not investigate whether a threshold Ψ_p exists for these processes to occur.

Changes in $E_{CO_2}(20)$ were clearly related to simulated changes in Ψ_p , but the response of $E_{CO_2}(20)$ lagged behind changes in Ψ_p (Figure 4.6c and d). A possible explanation is that when the CO₂ production by the living cells increases due to improved water status, its radial diffusion through the stem into the cuvette is delayed by the large resistance in the stem. A substantial restriction to radial gas movement in *Pinus strobus* L. branches was demonstrated by Eklund and Lavigne (1995).

As mentioned in the introduction, an explanation for daytime depressions in E_{CO_2} is that the flowing sap in the xylem of transpiring trees transports respiratory CO₂ away from the stem. These studies therefore link E_{CO_2} to f_s . Our study links daytime depressions in E_{CO_2} to changes in Ψ_p , and, hence, indirectly also to f_s : changes in Ψ_p reflect changes in the water status in the living stem tissues, which result from changes in f_s . Until now, the effect of f_s on turgor in the living stem tissues and, hence, on the stem growth rate and respiration has been ignored. With our dataset we could not distinguish between the effects of CO₂ transport in the xylem and those of turgor dynamics in the living stem tissues. Thus our work does not refute previous explanations; rather it points out that in addition, just as well as the idea that respired CO₂ is transported with the transpiration stream, the daily dynamics of Ψ_p and growth might explain daytime depressions in stem E_{CO_2} . Further studies need to be conducted, in which the effects of CO₂ transport in the xylem and those of turgor and growth dynamics in the living tissues external to

the xylem on stem E_{CO_2} are separated, and in which the relative importance of both processes on stem E_{CO_2} are investigated. It is possible that processes in the external tissues have a larger impact on stem E_{CO_2} than the CO_2 transport in the xylem. Some facts support this hypothesis. The cambium is quite impermeable to gases (Hook et al. 1972), which is demonstrated by the large difference in CO_2 concentrations between xylem and bark tissues: xylem CO_2 concentrations have been reported to be as high as 26% (MacDougal and Working 1933), while CO_2 concentrations in bark tissues are reported to be around 0.06 to 0.17% (Cernusak and Marshall 2000, Wittmann et al. 2006). Furthermore, Maier and Clinton (2006) measured xylem CO_2 concentrations and E_{CO_2} in young *Pinus taeda* L. tree stems during spring and found that, after partial removal of the canopy, xylem CO_2 concentrations increased, but there was no apparent change in stem E_{CO_2} . They assumed that during spring the cambium and phloem meristems likely respire at much higher rate than the xylem parenchyma and thus would be a major source of CO_2 in the stem.

However, it should be noted that also the xylem contains living cells (mainly ray parenchyma), which may be also sensitive to changes in turgor. The RCGro model only simulates changes in turgor of the living cells located in tissues external to the xylem. For small trees, the amount of living cells in the xylem is very small compared to the entire stem cross sectional area, because the ratio phloem/xylem volume is large. In these trees it seems very reasonable that external living stem tissues have a major impact on stem E_{CO_2} . In larger trees the xylem becomes more important compared to the other tissues, so that the amount of living xylem cells can surpass the number of living phloem and cambium cells. In that case, the contribution of respiring xylem cells to stem E_{CO_2} may become more important. This implies that for large trees the diurnal dynamics of turgor in the xylem living cells should also be quantified in order to reveal whether water deficit in xylem tissues can affect stem E_{CO_2} .

4.5 Conclusions

This study demonstrates that the loss of Ψ_p in the external living stem tissues during the daytime is quantitatively consistent with a slowing, or even cessation, of growth processes in these tissues. Since growth respiration is an important component in total stem respiration, daytime depressions in stem E_{CO_2} (i.e., lower compared with what would be expected from the exponential temperature function) can at least partially be explained by the restricted growth during the daytime. However, since stem E_{CO_2} also responded to changes in Ψ_p when the Ψ_p was still lower than the wall-yielding threshold value for growth, it was suggested that not only growth rate, but also the rate of maintenance metabolism

fluctuates daily, due to the daily dynamics in the water status in the living stem tissues.

This work did not aim at distinguishing between the effects of turgor and the effects of transport of dissolved CO₂ in the xylem. It rather wants to demonstrate that the daily dynamics of the water status in the living stem tissues might explain daytime depressions in stem E_{CO_2} just as well as the idea that the transpiration stream is transporting CO₂ in the xylem. It will be a challenge in further research to separate the effects of both processes and to unravel to what extent both processes might influence stem E_{CO_2} .

Chapter 5

Drought and the diurnal patterns of stem CO₂ efflux and xylem CO₂ concentration in young oak (*Quercus robur*)

Adapted from : Saveyn A, Steppe K and Lemeur R (2007) Drought and the diurnal patterns of stem CO₂ efflux and xylem CO₂ concentration in young oak (*Quercus robur*). *Tree Physiology* 27: 365 – 374

Abstract

Drought stress was imposed upon a young oak (*Quercus robur* L.) tree by interrupting the water supply for 9 days. The tree was placed in a growth chamber in which temperature and radiation patterns were kept constant. The effects of drought stress on the water and carbon status of the stem were examined by measuring stem sap flow rate, stem water potential, stem diameter variations, stem CO₂ efflux rate (E_{CO_2}) and xylem CO₂ concentration ($[CO_2]$). Before and after the drought stress, diurnal fluctuations in E_{CO_2} and $[CO_2]$ corresponded well with variations in stem temperature (T_{st}). Daytime depressions in E_{CO_2} did not occur. During drought stress, E_{CO_2} still responded to stepwise changes in temperature, but diurnal fluctuations in E_{CO_2} were no longer correlated with diurnal fluctuations in T_{st} , but became strongly correlated with diameter variations, exhibiting clear daytime depressions. The depressions in E_{CO_2} were likely the result of a reduction of the metabolic activity under a lowered daytime stem water status. Also xylem $[CO_2]$ showed clear daytime depressions under drought stress. When the tree was re-watered, E_{CO_2} and $[CO_2]$ exhibited a sharp increase, which coincided with the diameter increase. After the water supply, daytime depressions in E_{CO_2} and $[CO_2]$ disappeared and diurnal fluctuations in E_{CO_2} and $[CO_2]$ corresponded again with variations in T_{st} .

5.1 Introduction

Temperature (T) is an important environmental factor controlling respiration rates (Amthor 1989). Respiration rates of woody tissues are commonly measured as the CO_2 efflux rate (E_{CO_2}) from that tissue. In some cases, a good relationship has been found between measured E_{CO_2} and T (e.g., Paembonan et al. 1991, Maier et al. 1998); however, in other cases a relationship between measured E_{CO_2} and T was less clear or absent. Kakubari (1988) observed reductions in stem E_{CO_2} for *Fagus sylvatica* L. that did not correspond to fluctuations in stem temperature (T_{st}) or air temperature (T_{a}). In summer, Kaipainen et al. (1998) did not find any correlation between measured E_{CO_2} in stems of *Pinus sylvestris* L. and T_{a} , even when accounting for the possibility that T_{st} may lag the change in T_{a} .

Variation in sap flow rate may be responsible for the inconsistent relationship observed between T and stem E_{CO_2} (Martin et al. 1994). In part, CO_2 evolved by the respiring cells of the stem is dissolved in the sap and is transported with the transpiration stream instead of escaping through the bark (Negisi 1972, Ryan 1990, Sprugel 1990, Teskey and McGuire 2002). Furthermore, CO_2 originating from root or microbial respiration is transported with the transpiration stream and released at stem level (Levy et al. 1999, Teskey and McGuire 2007). In either case, measurement of E_{CO_2} may provide an erroneous estimate of stem respiration.

Water deficit in the stem tissue may also modify the relationship between T and stem E_{CO_2} . Wang et al. (2003) found that stem E_{CO_2} of *Larix gmelini* Rupr. trees was closely correlated with T_{st} in the morning but not in the afternoon, and suggested that this could be due to a greater stem water deficit in the afternoon. Lavigne (1987) observed lower stem E_{CO_2} of *Abies balsamea* (L.) Mill. trees on some afternoons compared with mornings, and suggested that this was caused by low stem water content, which temporarily reduced rates of synthesis of new structures and impaired normal maintenance processes. Hence, reduced growth respiration and maintenance respiration may at least partly explain midday depressions in stem E_{CO_2} . In that case, depressions in measured E_{CO_2} are an indication of actual depressions in respiration rates.

We are unaware of any investigations on how the diurnal pattern of E_{CO_2} of woody tissues is affected by a declining tissue water content induced by a gradually decreasing soil water potential. Therefore, our main objectives were to study the impact of soil water depletion on: (1) the diurnal pattern of stem E_{CO_2} and xylem CO_2 concentration, (2) the relationship between stem E_{CO_2} and T_{st} ; and (3) the relationship between stem E_{CO_2} and diurnal changes in stem diameter in a young *Quercus robur* L. tree.

5.2 Materials and methods

5.2.1 Plant material and experimental conditions

The experimental setup for this study is similar as described in Chapter 3 (Figure 3.1). At the end of February 2005, two 3-year-old oak (*Quercus robur* L.) trees, T1 and T2, previously grown outdoors, were planted in 50 l containers filled with a mix of sandy loam soil and potting mixture. The trees were placed in a growth chamber of 2 × 1.5 × 2 m (height × width × length) at Ghent University (51°3' N, 3°43' E), which allowed control of irradiance and air temperature. Light was supplied by fluorescent lamps ('TL'D 80, Philips Lighting NV, Brussels, Belgium), producing photosynthetic active radiation (PAR) of 473 μmol m⁻² s⁻¹ at the top of the trees during the 12-h daily photoperiod. Air temperature (T_a) was measured with a copper-constantan thermocouple (Omega, Amstelveen, Netherlands), installed at a height of 1.1 m between the trees. Relative humidity (RH) was measured with a capacitive RH sensor (Model HIH-3605-A, Honeywell, Freeport, IL, USA) at the same height of the thermocouple. Water potential of the soil (Ψ_{so}) was measured with tensiometers (Model SWT6, Delta-T, Cambridge, UK) at a depth of 20 cm. The trees were watered daily and fertilized monthly with a NPK plus micronutrient mix (Substral, Sint-Niklaas, Belgium). The experiment was started on 22 June 2005 (day 173) and ended on 13 July 2005 (day 194). At the beginning of the experiment the trees were 1.6 m high and the stem diameters at the soil surface were 19.2 and 17.8 mm, for T1 and T2, respectively. Water supply of tree T2 was stopped on day 176. The tree was re-watered on day 185. From day 186 on, the tree was watered daily again.

5.2.2 CO₂ efflux measurement

CO₂ exchange measurements were performed at 0.1 m above soil level on 13-cm long stem segments of trees T1 and T2. The stem segments were enclosed in air-tight cylindrical (6 cm diameter) acrylic cuvettes which were wrapped in several layers of aluminium foil and black tape. The cuvettes and the stem parts above and below the cuvette were covered with reflective bubble insulation to exclude all light from reaching the stem tissue so that cortical or wood photosynthesis was prevented. In order to minimize short-term fluctuations in CO₂ concentration, air from the growth chamber was pumped at a rate of 1 l min⁻¹ through the cuvettes via a 50 l buffer tank, dried (Model CG/G 73-4, Hartmann and Braun AG, Germany gas cooler operating at 4 °C) and analyzed for CO₂ concentration with an infrared gas analyzer (IRGA; Model Binos 100-4P, Fisher-Rosemount, Hasselroth, Germany), operated in differential configuration. The reference cuvette had the same dimensions as the measuring cuvettes but contained no stem segment. Signals from the IRGA were logged every 10 s for 4 min for each stem segment, and mean values were recorded by a data logger

(HP 34970A, Hewlett Packard, Palo Alto, CA, USA). CO₂ efflux rates (E_{CO_2}) were expressed per unit of stem surface area.

5.2.3 Stem diameter measurement

Variations in stem diameter (D) were measured with linear variable displacement transducers (LVDTs) (Model LBB, 375-PA-100, Schaevitz, Hampton, VA, USA) and a transducer bridge (Model 8C-35, Schaevitz, Hampton, VA, USA), placed 2 cm below the stem cuvettes, and attached to the stem with a stainless steel holder. Changes in D can be divided into an irreversible component related to tissue growth and a reversible component related to the changes in hydration of the extensible tissues (Kozlowski 1972). It is therefore possible to define a daily growth (DG), corresponding to the difference between two successive daily maximum values of D (just before onset of shrinkage) and a maximum daily shrinkage (MDS), corresponding to the difference between the maximum and minimum values of D during a day.

5.2.4 Sap flow measurement

Sap flow rates (f_s) were measured at the base of the stem with heat balance sap flow sensors (Model SGB16-WS, Dynamax Inc., Houston, TX, USA). The sensors were shielded from radiation by several layers of aluminium foil. Sheath conductance of the gauge was recalculated daily based on minimum predawn values between 0400 and 0700 h. The value for stem thermal conductance was taken from the literature: for woody stems a value of $0.42 \text{ W m}^{-1} \text{ }^\circ\text{C}^{-1}$ is considered appropriate according to Steinberg et al. (1989).

5.2.5 Measurement of stem water potential

Leaf water potential was measured daily with a pressure chamber (PMS, Corvallis, OR, USA) just before the lights were turned on. Because f_s was zero in the dark period, this value corresponds with the predawn stem water potential ($\Psi_{\text{st}}^{\text{p}}$). At 1400 h the water potential was measured on a leaf that was prevented from transpiration for 2 h by enclosing it in an aluminium envelope. Assuming zero sap flow in the petiole of the leaf, this value corresponds with the midday stem water potential ($\Psi_{\text{st}}^{\text{m}}$) (McCutchan and Shackel 1992).

5.2.6 Measurement of xylem CO₂ concentration

Xylem CO₂ concentration ($[\text{CO}_2]$; %) was measured *in situ* at the base of the stem of T1 and T2 using the microelectrode technique described by McGuire and Teskey (2002). To measure xylem $[\text{CO}_2]$ *in situ*, 4-mm diameter holes were drilled 7 mm deep into the xylem at approximately 1 cm below the stem cuvettes. Teflon tubes (3.5 mm inner diameter and 30 mm length) were tightly fitted into the

holes and the tubes were sealed to the trees using flexible putty adhesive (Terostat IX, Henkel, Heidelberg, Germany). A CO₂ microelectrode (Model MI-720, Microelectrodes Inc., Bedford, NH, USA) was inserted in the tube and putty adhesive was used to provide a gas-tight seal. The microelectrode measured the CO₂ concentration of the gas in the headspace of the hole. Due to a malfunction of the microelectrode, [CO₂] of tree T1 was only measured until day 182.

5.2.7 Temperature response of CO₂ efflux rate

Stem temperature (T_{st}) was measured with copper-constantan thermocouples inserted in the stem in 1-mm diameter holes with a depth of 7 mm, just below the stem cuvettes. To investigate temperature dependence of physiological variables, T_a was altered stepwise (in four steps of 1 h: 24 – 21 – 17 – 21 °C) during the dark period of day 176, 184 and 190 and during the light period of day 183 and 190.

Values of E_{CO_2} ($\mu\text{mol m}^{-2} \text{s}^{-1}$) were regressed against stem temperature T_{st} (°C) as:

$$E_{CO_2} = E_{CO_2}(20) \times Q_{10}^{\frac{T_{st}-20}{10}} \quad (5.1)$$

where $E_{CO_2}(20)$ is the CO₂ efflux rate at 20 °C, and Q_{10} is the relative increase in respiration rate with a 10 °C rise in T . Parameters $E_{CO_2}(20)$ and Q_{10} were estimated by ordinary least squares with Matlab 6.5 software (The Mathworks Inc., Natick, MA, USA).

5.2.8 Data logging

All signals from sensors and devices were logged at 10-s intervals with a data acquisition system (HP 34970A, Hewlett Packard, Palo Alto, CA, USA). Sensor signals were averaged over 4-min periods and recorded by a computer.

5.3 Results

5.3.1 Microclimatological variables

Mean T_{st} during the 20 day measurement period was $21.0 \text{ °C} \pm 0.3 \text{ °C}$ and $20.7 \pm 0.3 \text{ °C}$ for the control tree T1 and the treatment tree T2, respectively (Figure 5.1a). Mean Ψ_{so} of tree T1 was -0.004 MPa (Figure 5.1b). From day 176 onward Ψ_{so} of tree T2 gradually decreased to a minimum value of -0.1 MPa on day 185.

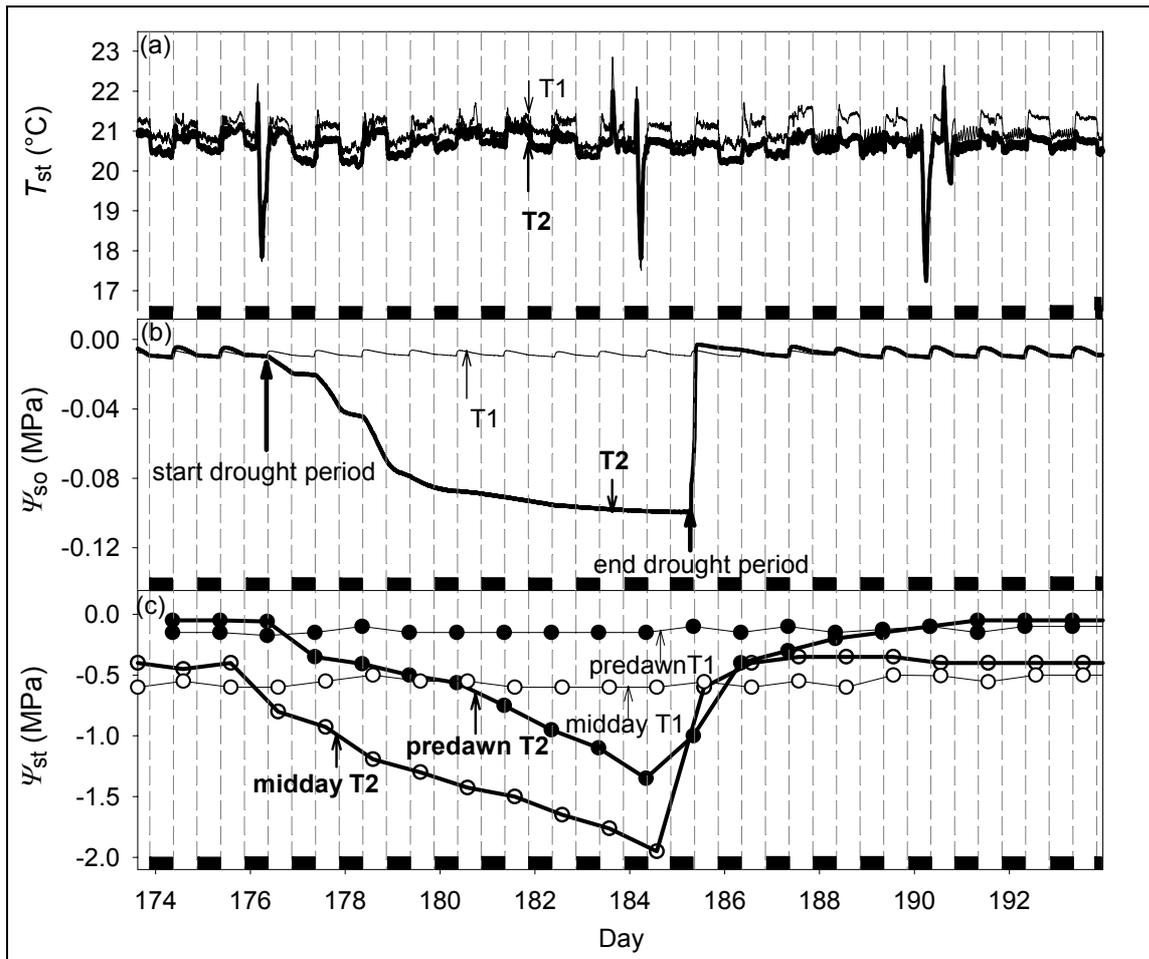


Figure 5.1: For the period day 173-194: (a) pattern of stem temperature (T_{st}) of trees T1 and T2; (b) soil water potential (Ψ_{so}); (c) predawn and midday stem water potential (Ψ_{st}). Thin lines denote tree T1 (control tree), bold lines denote tree T2 (treatment tree). Black boxes indicate dark periods.

5.3.2 Stem water potential

Both Ψ_{st}^p and Ψ_{st}^m of tree T1 remained quite constant during the entire measurement period. Mean Ψ_{st}^p was -0.13 MPa and mean Ψ_{st}^m -0.56 MPa. In the pre-drought period ($\Psi_{so} > -0.01$ MPa), Ψ_{st} of tree T2 showed daily variations with predawn values of -0.05 MPa and midday values of -0.4 MPa (Figure 5.1c). After water supply was stopped, Ψ_{st}^p and Ψ_{st}^m decreased significantly, with Ψ_{st}^m reaching -2 MPa on day 184. Complete recovery of the water status was achieved shortly after re-watering the tree.

5.3.3 Sap flow rate

Daily f_s of the control tree T1 was more or less constant throughout the measurement period (Figure 5.2a), with a mean f_s during the light periods of 23 ± 1 g h⁻¹. In the pre-drought period, mean f_s during the light periods of tree T2

was $31.1 \pm 0.8 \text{ g h}^{-1}$ (Figure 5.2a). f_s of T2 gradually decreased from day 179, reaching a minimum value of 9.8 g h^{-1} on day 184. When the tree was re-watered daily f_s started to increase, but it took 5 days to obtain a stable f_s during the light period. Mean f_s for the period day 190-194 was $24.4 \pm 0.4 \text{ g h}^{-1}$.

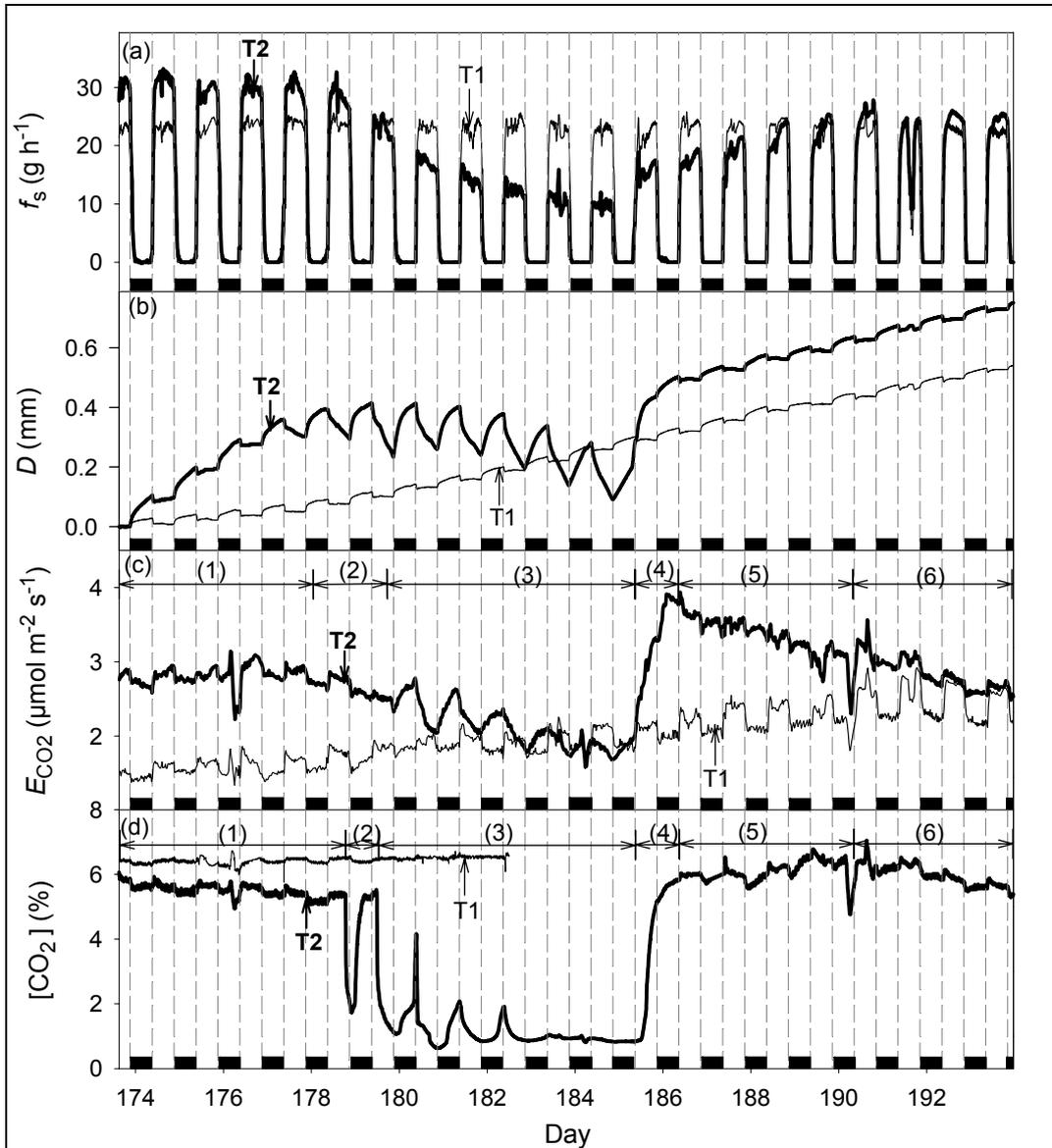


Figure 5.2: For the period day 173-194: (a) sap flow rate (f_s) at the stem base; (b) stem diameter variations (D); (c) stem CO₂ efflux rate (E_{CO_2}); (d) xylem CO₂ concentration ($[CO_2]$). Values of D were set to zero at the beginning of the measurement period. Thin lines denote tree T1 (control tree), bold lines denote tree T2 (treatment tree). Black boxes indicate dark periods.

5.3.4 Stem diameter variations

Figure 5.2b shows the time course of the stem diameter variations D , in T1 and T2 as measured by the two LVDTs. Variations in D reflect both irreversible growth and reversible changes in stem hydration. Growth was characterized by DG, and changes in stem hydration by MDS. For tree T1 MDS of $18 \pm 2 \mu\text{m}$ was

superimposed on a continuous growth curve. Mean DG of tree T1 was $25 \pm 8 \mu\text{m day}^{-1}$. In the pre-drought period, Tree T2 had an MDS of $21 \pm 2 \mu\text{m}$. During the pre-drought period, stem shrinkage was observed for 1 h after the lights were turned on and then D exhibited an increasing trend during the rest of the light period, indicating that irreversible growth occurred. In response to drought, MDS increased gradually, reaching a maximum value of $225 \mu\text{m}$ on day 184. From day 177, D showed a continuous decrease during the light period, indicating that, from day 177, irreversible growth no longer occurred during the light period. After the drought, mean MDS of tree T2 was $17 \mu\text{m}$, which is similar to the pre-drought value. Reversible changes in stem hydration showed a similar pattern after the drought as in the pre-drought period, i.e., stem shrinkage for 1 h after the lights were turned on and a constant or slightly increasing value of D during the rest of the light period, indicating that irreversible growth occurred. Mean DG of tree T2 in the pre-drought period was $93 \pm 0.3 \mu\text{m day}^{-1}$ and it became zero on day 179.

From day 179 onward, stem water reserves of tree T2 were no longer fully replenished during the dark period and, hence, a negative DG was observed. After the drought period mean DG was $33 \pm 4 \mu\text{m day}^{-1}$.

5.3.5 CO_2 efflux rate

During the pre-drought period, the mean DG of tree T1 was $25 \mu\text{m day}^{-1}$, versus $93 \mu\text{m day}^{-1}$ in tree T2 and stem E_{CO_2} of tree T2 was about twice that of T1 (Figure 5.2c). E_{CO_2} of tree T1 showed diurnal fluctuations that closely corresponded with T_{st} (cf. Figures 5.2c and 5.1a). For tree T2, six different periods could be distinguished in the time course of E_{CO_2} (Figure 5.2c). (Period 1) From day 173 until day 178, E_{CO_2} of trees T2 and T1 showed a similar pattern. Diurnal fluctuations in E_{CO_2} corresponded closely with fluctuations in T_{st} (Figure 5.3a). (Period 2) For tree T2, day 178-179 was a transitional period in which fluctuations in E_{CO_2} were poorly correlated with fluctuations in T_{st} (Figure 5.3d). On day 179, there was no relationship between E_{CO_2} and T_{st} . At that time Ψ_{so} was -0.085 MPa and Ψ_{st} was about -1.3 MPa . (Period 3) From day 180 until tree T2 was re-watered on day 185, E_{CO_2} showed diurnal fluctuations that were unrelated to changes in T_{st} (Figure 5.3g). During this period, fluctuations in E_{CO_2} were more closely correlated with variations in D than in T_{st} (Figure 5.3h). (Period 4) When water was given on day 185, E_{CO_2} sharply increased and reached a maximum value in the dark period of day 186. This increase coincided with a sharp increase in D (Figure 5.3k). (Period 5) During the period day 186-189, there was still no clear correlation between fluctuations in E_{CO_2} and T_{st} (Figure 5.3m) and the correlation between D and E_{CO_2} was also poor (Figure 5.3n).

Stem CO_2 efflux and xylem CO_2 concentration under drought stress

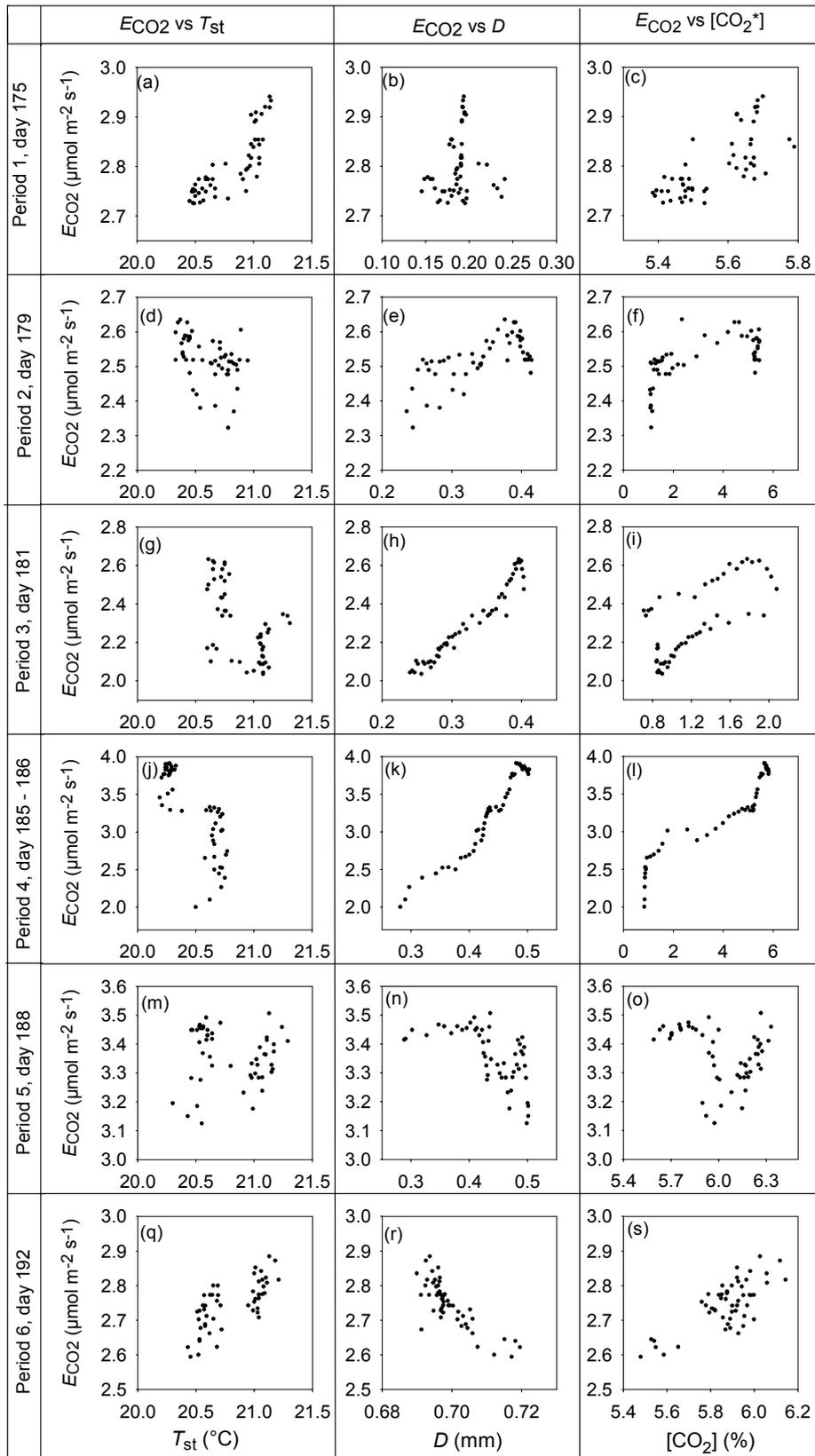


Figure 5.3: Relationships between stem CO_2 efflux rate (E_{CO_2}) and stem temperature (T_{st}), stem diameter D and xylem CO_2 concentration ($[CO_2]$) for tree T2 on six days in the six different periods as indicated in Figures 5.2c and 5.2d. Days are from 0000 h to 0000 h, except for Period 4, where a time period from 0900 h (moment of water supply) until 0900 h the next day was chosen.

(Period 6) From day 190 on, fluctuations in E_{CO_2} again corresponded with fluctuations in T_{st} (Figure 5.3q); however, because E_{CO_2} continued to show an overall declining trend (Figure 5.2c), the correspondence was less clear than during the pre-drought period (Period 1; Figure 5.3a).

5.3.6 Temperature response of CO_2 efflux rate

In the pre-drought period (Period 1), diurnal fluctuations in stem E_{CO_2} of tree T2 corresponded closely with fluctuations in T_{st} (Figure 5.3a). Values of the parameters $E_{\text{CO}_2(20)}$ and Q_{10} for T2 were estimated based on data of day 176, when T_a was altered stepwise. A plot of E_{CO_2} as a function of T_{st} exhibited hysteresis following a counter clockwise time course. The hysteresis was not apparent when E_{CO_2} was plotted against T_{st} 28 min earlier (prior temperature) (Figure 5.4). An exponential regression between E_{CO_2} and lagged T_{st} revealed a high coefficient of determination ($R^2 = 0.89$). Estimated Q_{10} and $E_{\text{CO}_2(20)}$ are given in Table 5.1. Respiration rates calculated using Equation 5.1 and parameters estimated from day 176 (Model 1, Figure 5.5) corresponded closely with measured E_{CO_2} for the period day 173-177.

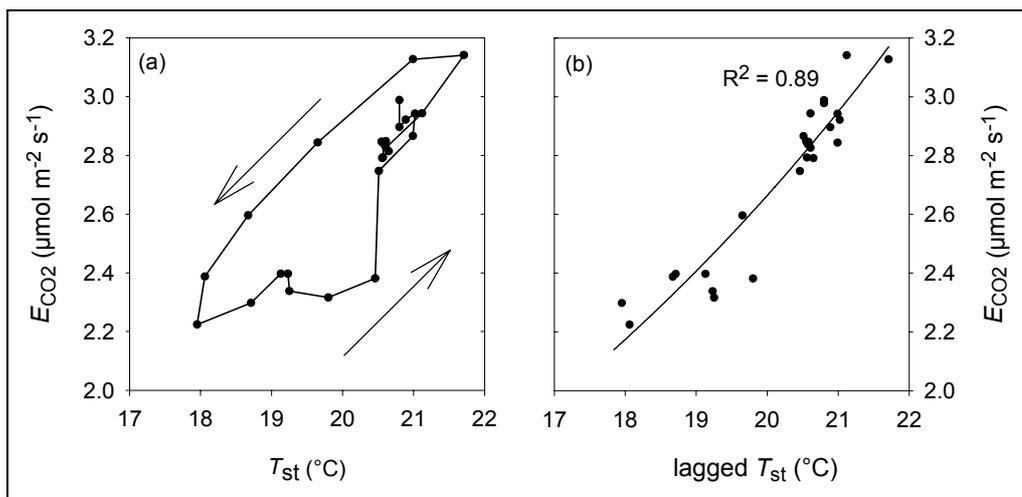


Figure 5.4: Stem CO_2 efflux rates (E_{CO_2}) from the stem segment of tree T2 on day 176 (dark period) versus (a) current stem temperature (T_{st}) and (b) lagged stem temperature. The coefficient of determination (R^2) for the Q_{10} model (Equation 5.1) fit to the data is also given. Arrows show the time course of the hysteresis phenomenon.

During the drought period (Period 3), E_{CO_2} showed diurnal fluctuations which were not related to fluctuations in T_{st} (Figure 5.3g). However, when T_a was altered stepwise on day 183-184, these changes were still notable in the course of E_{CO_2} (Figure 5.2c). For day 183-184, a temperature lag of 16 min provided the best fit of the model to current E_{CO_2} , based on the coefficient of determination. Estimated Q_{10} and $E_{\text{CO}_2(20)}$ are given in Table 5.1. Respiration rates predicted from lagged T_{st} using Equation 5.1 with parameters estimated from day 183-184 (Model 2) are shown in Figure 5.5. Although the fit was good for the period in

which the temperature steps were imposed, predicted respiration rates did not correspond with measured E_{CO_2} for the rest of the period day 180-185.

Table 5.1: Characteristics of the response of E_{CO_2} to T_{st} . Q_{10} is the increase in respiration rate for a 10 °C increase in temperature, $E_{CO_2}(20)$ is the CO₂ efflux rate at 20 °C (Equation 5.1); and R^2 is the coefficient of determination for the exponential regression between E_{CO_2} and T_{st} . Standard errors of the mean are given in parentheses.

Period	Lag period for best fit (min)	Q_{10}	$E_{CO_2}(20)$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	R^2
Day 176	28	2.76 (0.12)	2.66 (0.019)	0.89
Day 183-184	16	1.94 (0.09)	1.83 (0.008)	0.90
Day 190	36	2.45 (0.10)	2.95 (0.012)	0.91

After the drought from day 190 onward (Period 6), diurnal fluctuations in E_{CO_2} corresponded again with fluctuations in T_{st} (Figure 5.3q). On day 190 T_a was altered stepwise. A time lag for temperature of 36 min provided the best fit (Equation 5.1) to the data, based on the coefficient of determination. Estimated Q_{10} and $E_{CO_2}(20)$ are given in Table 5.1. Respiration rates calculated with Equation 5.1 and parameters estimated from day 190 (Model 3, Figure 5.5) followed the same diurnal trend as measured E_{CO_2} for the period day 190-194. However, during this period, E_{CO_2} still exhibited an overall declining trend and therefore, measured E_{CO_2} did not completely coincide with calculated respiration rates.

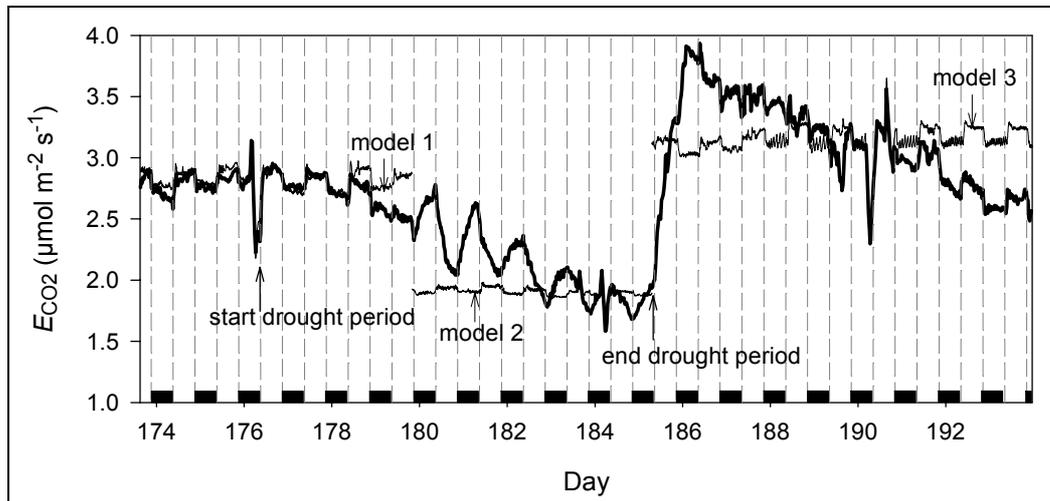


Figure 5.5: Measured stem CO₂ efflux rates (E_{CO_2}) (bold line) and predicted respiration rates (thin lines) based on Equation 5.1 for tree T2, for the period day 173-194. Parameters Q_{10} and $E_{CO_2}(20)$ were estimated based on data of day 176 for Model 1, on data of day 183-184 for Model 2 and on data of day 190 for Model 3 (Table 5.1). Black boxes correspond to the dark periods.

5.3.7 Relationship between CO₂ efflux rate and diameter variations

In the pre-drought period (Period 1), fluctuations in E_{CO_2} appeared to be in opposite phase with fluctuations in D : lights off coincided with an increase in D and a decrease in E_{CO_2} and the onset of lights was immediately followed by a decrease in D and an increase in E_{CO_2} (cf. Figures 5.2c and 5.2b). However, when E_{CO_2} was normalized to a common temperature using Equation 5.1 ($T_{\text{st}} = 21\text{ }^\circ\text{C}$, $Q_{10} = 2.76$), E_{CO_2} showed little variation and was no longer negatively correlated with D .

Under drought, from Period 3 on, diurnal fluctuations in E_{CO_2} were strongly positively correlated with fluctuations in D (Figure 5.3h). When E_{CO_2} was plotted against D for the period day 180 - 185, a clear exponential relationship ($R^2 = 0.93$) was observed (Figure 5.6). When tree T2 was re-watered on day 185 (Period 4), E_{CO_2} and D both increased sharply and the variables were strongly positively correlated (Figure 5.3k).

After the drought period, from day 186 on (Period 5), no correlation between E_{CO_2} and D was observed (Figure 5.3n), and from day 190 onward (Period 6), E_{CO_2} appeared to be negatively correlated with D (Figure 5.3r). However, when E_{CO_2} was normalized to a common temperature with Equation 5.1 ($T_{\text{st}} = 21\text{ }^\circ\text{C}$, $Q_{10} = 2.45$), this correlation was no longer apparent.

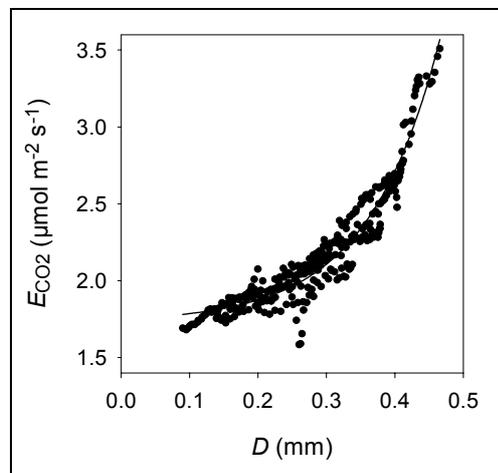


Figure 5.6: Stem CO₂ efflux rate (E_{CO_2}) of T2 as a function of stem diameter (D) for the period day 180-185. The solid line represents the least squares exponential regression of the relationship between E_{CO_2} and D . R^2 of the exponential regression between measured E_{CO_2} and D is 0.93.

5.3.8 Xylem CO₂ concentration

Xylem [CO₂] of tree T1 was nearly constant, with only small variations which corresponded well with variations in T_{st} : higher values of [CO₂] occurred during the light period together with higher values of T_{st} (Figure 5.2d versus 1a). Xylem and E_{CO_2} were well correlated for tree T1 (cf. Figures 5.2d and 5.2c). For tree T2

again six periods could be distinguished (Figure 5.2d). (Period 1) From day 173 until day 177, the daily course of [CO₂] followed a similar trend as in tree T1. Fluctuations in [CO₂] corresponded with fluctuations in T_{st} . (Period 2) On day 178, at 7 pm, a first abrupt drop in xylem [CO₂] was observed at 1900 h. At the end of the photoperiod, an increase was observed until the original value was reached. During the rest of the dark period and a part of the light period on day 179, fluctuations in [CO₂] of T2 corresponded with fluctuations in T_{st} . (Period 3) From day 180 until day 185, no relationship between [CO₂] and T_{st} was observed. Xylem [CO₂] was more closely correlated with the dark-light pattern: lights off coincided with an increase in [CO₂] and the onset of lights was followed by a decrease. In this period, the magnitude of the increase of during the dark period gradually diminished: on day 180, [CO₂] increased during the dark period, but the value at the end was only 69% of the original mean [CO₂] and on day 181, 182, 183 and 184 the peak was further decreased to 23, 20, 2 and 2% of the original value, respectively. (Period 4) When tree T2 was re-watered on day 185, [CO₂] sharply increased. (Period 5) During the period day 186-189, [CO₂] remained constant or exhibited a slightly declining trend during the light periods, while during the dark periods [CO₂] showed first a decrease for 2 h and then an increase until the end of the dark period. In this period, fluctuations in [CO₂] started to correspond again with fluctuations in T_{st} . (Period 6) From day 190 on, diurnal fluctuations in [CO₂] corresponded closely with fluctuations in T_{st} .

5.4 Discussion

5.4.1 Stem diameter variations

Variations in D reflect both daily reversible shrinkage and swelling patterns due to variations in stem water potential (Klepper et al. 1971, Garnier and Berger 1986) and irreversible growth patterns. A cell grows when the water influx into the cell is accompanied by cell wall extension. According to the most widely used model of cell expansion developed by Lockhart (1965), cell expansion depends on cell turgor pressure, cell wall extensibility and a threshold turgor pressure at which wall yielding occurs. Turgor pressure must be above this threshold value for the cell to grow. This idea has recently been incorporated in a mathematical model linking tree sap flow dynamics to daily stem diameter fluctuations and radial stem growth (Steppe et al. 2006). The model demonstrates that radial stem growth of trees occurs only when turgor exceeds a wall-yielding threshold value. Consistent with the models, DG in our study showed a declining trend and became zero 3 days after water supply of tree T2 was stopped. Besides water, carbon is also necessary for cells to grow. During a drought the supply of carbohydrates declines because of reduced photosynthesis. Hence, cessation of growth can be a direct consequence of the lowered turgor pressure or an indirect consequence of the limited carbohydrate supply, or both (Daudet et al. 2005).

5.4.2 CO_2 efflux rate

Studies on the effects of soil water depletion on the respiration of woody tissues of trees are scarce. Moreover, we are unaware of any studies on how the diurnal pattern of E_{CO_2} of woody tissues is affected by a gradually decreasing soil water potential. In this study, we found a clear phase shift in the daily course of stem E_{CO_2} during an imposed drought.

In the pre-drought period, diurnal fluctuations in E_{CO_2} were well correlated with the diurnal pattern of T_{st} (cf. Figures 5.2c and 5.1a). Several researchers have suggested that during the day, when the tree is transpiring, the transpiration stream in the xylem becomes a sink for respired CO_2 , resulting in a reduction of stem E_{CO_2} (Negisi 1972, Ryan 1990, Sprugel 1990, Teskey and McGuire 2002). Daytime reductions in stem E_{CO_2} were not observed, so in this case stem E_{CO_2} was not visibly affected by CO_2 transport in the xylem.

During the drought, E_{CO_2} showed daytime reductions, most likely because stem tissue metabolism decreased as a result of tissue dehydration during the daytime. This can be deduced from the finding that variations in E_{CO_2} were synchronized with variations in D (cf. Figures 5.2c and 5.2b). Another option is that the flowing xylem sap transported respiratory CO_2 away from the site of E_{CO_2} measurement. However, if true, it follows that the higher f_s , the greater the daytime depression. According to this interpretation, depressions in stem E_{CO_2} should be greatest under pre-drought conditions compared with the drought period because of higher f_s in the pre-drought period, which was not the case.

When tree T2 was re-watered, a sharp increase in E_{CO_2} was observed (Figure 5.2c). If sap flow is a sink for CO_2 (Negisi 1972), it would be expected that with the sudden increase in f_s (Figure 5.2a) the CO_2 accumulated within the stem would be carried away by the sap stream causing a depression in E_{CO_2} . However, we observed the opposite: the increase in f_s coincided with a sharp increase in E_{CO_2} (Figure 5.2c). Nevertheless, from our observations it cannot be concluded that sap flow was without effect on E_{CO_2} , as increased root and soil microbial metabolism after re-watering might have contributed CO_2 to the transpiration stream. Hence, the large pulse in E_{CO_2} after re-watering may have been the result of both increased metabolism in the local stem tissue and increased transport of CO_2 originating from root and soil microbial metabolism.

5.4.3 Temperature response of CO_2 efflux rate

During the pre-drought period, the diurnal pattern of E_{CO_2} was strongly determined by the pattern of T_{st} (Figure 5.3a). When T_a was altered stepwise (day 176), the relationship between T_{st} and E_{CO_2} had a good fit to an exponential equation when a time lag was taken into account (Figure 5.4). Hysteresis

between E_{CO_2} and T_{st} has been reported in several studies. Lavigne et al. (1996) found that a stem temperature lag of 1.75 h provided the best relationship with E_{CO_2} in balsam fir trees of 10 to 60 year old. Bosc et al. (2003) applied lag values up to 50 min to obtain a better fit between branch temperature and data of branch E_{CO_2} in adult *Pinus pinaster* Aiton. trees. The justification for such a procedure is that a delay can be assumed to exist between CO₂ production in the living tissues and CO₂ efflux at the organ surface because of the high resistance of the cambium and the bark (Eklund and Lavigne 1995). The Q_{10} value in the pre-drought period was 2.76, which is at the high end of the range of reported values for various tree species. For example, Edwards and Hanson (1996) found a Q_{10} of 2.4 for stems of *Quercus alba* L. and *Quercus prinus* L.

Three days after the imposition of drought, the diurnal pattern of E_{CO_2} was no longer correlated with the pattern of T_{st} (Figure 5.3g). Nevertheless, from the stepwise alterations of T_{a} on day 183-184, it was apparent that E_{CO_2} continued to respond to changes in T_{st} . However, Q_{10} was lower than during the pre-drought period (Table 5.1), reflecting reduced metabolic activity of the living cells.

Five days after re-watering, diurnal fluctuations in E_{CO_2} were again correlated with fluctuations in T_{st} (Figure 5.3q). Q_{10} was 2.45, which is higher than during the drought period, but slightly lower than in the pre-drought period. Hence, metabolic activity of the living cells was lower than in the pre-drought period. The mean DG was lower after the drought than before (33 versus 93 $\mu\text{m day}^{-1}$), suggesting that tree T2 had not yet completely recovered from the drought.

5.4.4 Relationship between CO₂ efflux rate and diameter variations

In the pre-drought period, irreversible growth was observed both during the light and dark periods (Figure 5.2b), indicating that turgor pressure in the living stem tissues was always higher than the threshold value necessary for cells to grow (Lockhart 1965). During the pre-drought period, E_{CO_2} was not correlated with D (Figure 5.3b). Because variations in D reflect variations in stem water status, rates of growth and maintenance processes were not visibly affected by stem water status during the pre-drought period.

During the drought, irreversible growth was no longer observed (Figure 5.2b). The strong correlation of E_{CO_2} with D during that period (Figure 5.6) demonstrates that E_{CO_2} became highly dependent on stem water status. When the tree was re-watered, a sharp increase of E_{CO_2} was observed, which coincided with the sharp increment of D due to re-hydration of the stem. From the strong correlation between E_{CO_2} and D it can be deduced that the temperature-independent variations in E_{CO_2} during the drought-period and at the moment of re-watering were the result of variations in rates of growth and maintenance processes following variations in water status. Therefore, our results agree with the view

that, during the day, water deficit causes a reduction in the rates of growth and maintenance processes and, hence, in respiration rates (Lavigne 1987, Wang et al. 2003). When the stress is (partly) alleviated, because of replenishment of the water reserves at night or because of water supply after a drought, E_{CO_2} increases as a result of an increase in the rate of maintenance and/or growth processes. Daudet et al. (2005) also found that E_{CO_2} in potted hybrid walnut (*Juglans nigra* × *J. Regia* cv. NG38) trees increased at night, and concluded that variations in E_{CO_2} reflect variations in the local respiration rate and must be attributed to metabolic changes. It was also reported for the herbaceous species *Sorghum bicolor* (L.) Moench that whole-plant-respiration increased dramatically on the first day after re-watering (Richardson and McCree 1985). Amthor (1994) suggests that this response is due to rapid growth and high growth respiration rates following stress alleviation and/or high maintenance respiration rates for rapid protein resynthesis and other cell repair processes. These studies and our results indicate that tree water status has an important influence on stem respiration rates, particularly when water availability is limited.

5.4.5 CO_2 efflux rate versus xylem CO_2 concentration

Teskey and McGuire (2002) found that diurnal fluctuations in xylem $[CO_2]$ corresponded closely to diurnal changes in stem E_{CO_2} . In this study xylem followed more or less the same pattern as E_{CO_2} in the pre-drought period (Figure 5.3c), but the correlation became less clear during the early drought period (Period 2, Figure 5.3f) and in the transitional period after re-watering (Period 5, Figure 5.3o). During the drought, E_{CO_2} and $[CO_2]$ were positively correlated, but a large time-lag between these variables was observed (Period 3, Figure 5.3i). However, it remains unclear why xylem $[CO_2]$ sometimes differed from E_{CO_2} . It is possible that E_{CO_2} is not closely related to processes occurring in the xylem because lateral diffusion of air in the xylem towards the atmosphere is restricted by a high resistance located at the xylem and cambial layers (Eklund and Lavigne 1995). If so, E_{CO_2} is mainly determined by respiration rates of the cells located in the cambium and phloem and sudden changes in xylem $[CO_2]$ may not have a direct influence on E_{CO_2} . After the drought period (Period 6, Figure 5.3s), E_{CO_2} and $[CO_2]$ were again positively correlated.

5.5 Conclusions

In a well-watered oak tree, diurnal fluctuations in stem E_{CO_2} and xylem $[CO_2]$ corresponded closely with fluctuations in T_{st} and daytime depressions in E_{CO_2} did not occur. When soil water availability became limited, clear daytime depressions in E_{CO_2} were observed. During that period, diurnal fluctuations in E_{CO_2} were more closely related to fluctuations in D than to fluctuations in T_{st} ; however, although the relation with stem D fluctuations became dominant, even during severe

drought stress E_{CO_2} continued to respond to stepwise alterations in T_a . Daytime depressions in E_{CO_2} during the drought likely resulted from the reduced rates of metabolic activity (growth and maintenance processes), which were in turn caused by the decrease in daytime stem water status. Q_{10} of stem E_{CO_2} was found to be lower during drought than in the pre-drought period, confirming the reduced metabolic activity of the living cells. Xylem $[\text{CO}_2]$ also showed clear daytime depressions in response to drought. When the tree was re-watered, E_{CO_2} and $[\text{CO}_2]$ showed sharp increases that coincided with the increase in D . After re-watering, daytime depressions in E_{CO_2} and $[\text{CO}_2]$ disappeared and the variables were again positively correlated with T_{st} . A notable finding is that xylem $[\text{CO}_2]$ was not always strictly correlated with E_{CO_2} . Large diffusion resistances presented by cambium and xylem and a predominant contribution of processes occurring in cambium and phloem to E_{CO_2} may have caused this decoupling.

Chapter 6

Resistance to radial CO₂ diffusion contributes to between-tree variation in CO₂ efflux rates of *Populus deltoides* stems

Adapted from : Steppe K, Saveyn A, McGuire MA, Lemeur R and Teskey RO (2007) Resistance to radial CO₂ diffusion contributes to between-tree variation in CO₂ efflux rates of *Populus deltoides* stems. *Functional Plant Biology* 24: 785 - 792

Abstract

CO₂ efflux rates of stems and branches are highly variable among and within trees and across stands. Scaling factors have only partially succeeded in accounting for the observed variations. In this study, the resistance to radial CO₂ diffusion was quantified for tree stems of an eastern cottonwood (*Populus deltoides* Bartr. ex Marsh.) clone by direct manipulation of the CO₂ concentration ([CO₂]) of xylem sap under controlled conditions. Tree-specific linear relationships between stem CO₂ efflux rates (E_{CO_2}) and xylem [CO₂] were found. The resistance to radial CO₂ diffusion differed 6-fold among the trees and influenced the balance between the amount of CO₂ retained in the xylem versus that which diffused to the atmosphere. Therefore, the hypothesis was put forward that variability in the resistance to radial CO₂ diffusion might be an overlooked cause for the inconsistencies and large variations in woody tissue E_{CO_2} . It was found that transition from light to dark conditions caused a rapid increase in E_{CO_2} and xylem [CO₂], both in manipulated trees and in an intact tree with no sap manipulation. This resulted in an increased resistance to radial CO₂ diffusion, at least for trees with relatively small daytime resistances. Stem diameter changes measured in the intact tree supported the idea that higher actual respiration rates occurred during the dark period due to higher metabolism in relation to an improved water status and higher turgor pressure.

6.1 Introduction

CO₂ efflux rates of stems and branches (E_{CO_2}) are quite variable among and within trees and across stands. For instance, large variations in efflux have been found within trees, with E_{CO_2} in the tree crown being 19-42 times greater than at the base of the stem (Damesin et al. 2002). Sprugel (1990) reported 10- to 40-fold differences in E_{CO_2} of stems and branches of 30-year-old *Abies amabilis* (Dougl.) Forbes trees. Variation among tree species is also substantial. For example, E_{CO_2} differed by 100% between *Platanus occidentalis* L. and *Liquidambar styraciflua* L. stems of similar size (Teskey and McGuire 2005). Similarly, E_{CO_2} was reported to differ by approximately 100-125% between stands of *Abies balsamea* (L.) Mill. (Lavigne et al. 1996) and *Picea mariana* (Mill.) B.S.P. (Lavigne and Ryan 1997).

The causes of this high variability have been difficult to isolate (Teskey and McGuire 2007). In quest of reliable predictors to account for the variability, several methods have been proposed for expressing temperature-corrected woody tissue E_{CO_2} , including stem surface area (Kinerson 1975), sapwood volume (Ryan 1990, Sprugel 1990) and tissue nitrogen concentration (Ryan 1991a). Contradictory results have often been reported (Ryan 1990, Stockfors and Linder 1998, Maier 2001, Bowman et al. 2005) and, hence, none of the proposed methods are able to fully account for the observed variation in measured E_{CO_2} .

These difficulties may suggest an inadequate understanding of the physiological processes and physical factors within stems that regulate CO₂ production and/or influence the radial diffusion of CO₂ through stems and branches into the atmosphere (Bowman et al. 2005). Recently, evidence has indicated that a portion of stem-respired CO₂ can dissolve in sap and move upward in the transpiration stream rather than escape to the atmosphere (Teskey and McGuire 2002, McGuire et al. 2007). Additionally, a portion of respired CO₂ originating elsewhere (in soil, roots or lower in the stem) can dissolve in sap and be transported to the site of efflux measurement, where it can escape to the atmosphere (Levy et al. 1999, Teskey and McGuire 2007). Therefore, factors affecting rates of transpiration can strongly influence the amount of CO₂ diffusing into the atmosphere, which might explain at least part of the variability observed among CO₂ efflux studies (Teskey and McGuire 2007, McGuire et al. 2007). Another factor that strongly influences the radial diffusion of respiratory CO₂ to the atmosphere is the resistance exerted by the xylem, cambium and bark layers (Eklund and Lavigne 1995). Although often ignored, differences in resistance might explain some of the inconsistencies observed in woody tissue E_{CO_2} measurements.

Previous direct manipulations of the CO₂ concentration ([CO₂]) of xylem sap have shown that stem E_{CO_2} is linearly related to xylem sap [CO₂] (Teskey and McGuire 2002, 2005). In this study, we experimentally manipulated stem xylem [CO₂] under controlled environmental conditions in a clone of *Populus deltoides* Bartr. ex Marsh. to further investigate the relationship between xylem [CO₂] and stem E_{CO_2} . The objective was to quantify the resistance to radial CO₂ diffusion for individual tree stems and to examine its variability and its effects on the variability of stem E_{CO_2} measurements.

6.2 Materials and methods

6.2.1 Plant material

Controlled experiments were conducted in a growth chamber (Model GC36, Environmental Growth Chambers, Chagrin Falls, OH, USA) at the Warnell School of Forestry and Natural Resources (WSFNR) of the University of Georgia near Athens, Georgia in October 2006 (Figure 6.1). Measurements were made on four 3-year-old trees of an eastern cottonwood (*Populus deltoides* Bartr. ex Marsh.) clone (Clone 98, Meadwestvaco Corp, Paducah, KY, USA) with diameters between 26.5 – 27.9 cm and heights of 2 – 3 m. The trees were grown in soilless media (4M Mix, Conrad Fafard Inc, Agawam, MA, USA) in 200 l containers in the greenhouse facilities of WSFNR and moved to the growth chamber immediately prior to beginning the experiment.

6.2.2 Measurements

Stem CO₂ efflux to the atmosphere (E_{CO_2}) was measured on 5-cm long stem segments using a fan-stirred cylindrical, opaque, polyvinyl chloride cuvette that completely surrounded the stem at 0.5 m above the soil. Using an opaque cuvette and covering the stem parts above and below the cuvette with reflective bubble insulation prevented cortical and wood photosynthesis. The bark of the *Populus deltoides* clone was smooth and did not require removal of loose bark or fissures to achieve an adequate seal between the cuvette and the bark. Closed-cell foam gaskets and hose clamps were used to fit the cuvette to the stem at both ends. Flexible putty adhesive (Qubitac, Qubit Systems, Kingston, Ontario, Canada) was applied to seal small gaps that typically occurred between the foam gaskets and the stem. Compressed air of known, near-ambient CO₂ concentration (388 ppm) was supplied to the cuvette at 0.5 l min⁻¹ using a mass flow controller (Model FMA 5514, Omega Engineering, Stamford, CT, USA). Air leaving the cuvette was dried over a column of magnesium perchlorate and its CO₂ concentration was measured with an infrared gas analyser (IRGA) (Model 7000, Li-Cor Inc, Lincoln, NE, USA) in an open flow configuration. E_{CO_2} (μmol m⁻³ s⁻¹) was calculated as:

$$E_{\text{CO}_2} = (f_a/V) \times \Delta[\text{CO}_2] \quad (6.1)$$

where f_a is the rate of air flow (mol s^{-1}) through the cuvette, V is the sapwood volume of the stem segment (m^3) and $\Delta[\text{CO}_2]$ is the difference in $[\text{CO}_2]$ of air flowing into and out of the cuvette ($\mu\text{mol mol}^{-1}$). In addition, E_{CO_2} was expressed per stem surface area ($\mu\text{mol m}^{-2} \text{s}^{-1}$) in order to be able to calculate the resistance (R) to radial CO_2 diffusion (s m^{-1}) according to Fick's first law:

$$R = \frac{\Delta[\text{CO}_2]}{E_{\text{CO}_2}} \quad (6.2)$$

where $\Delta[\text{CO}_2]$ is the difference in CO_2 concentration between the xylem and the air in the stem cuvette ($\mu\text{mol m}^{-3}$).

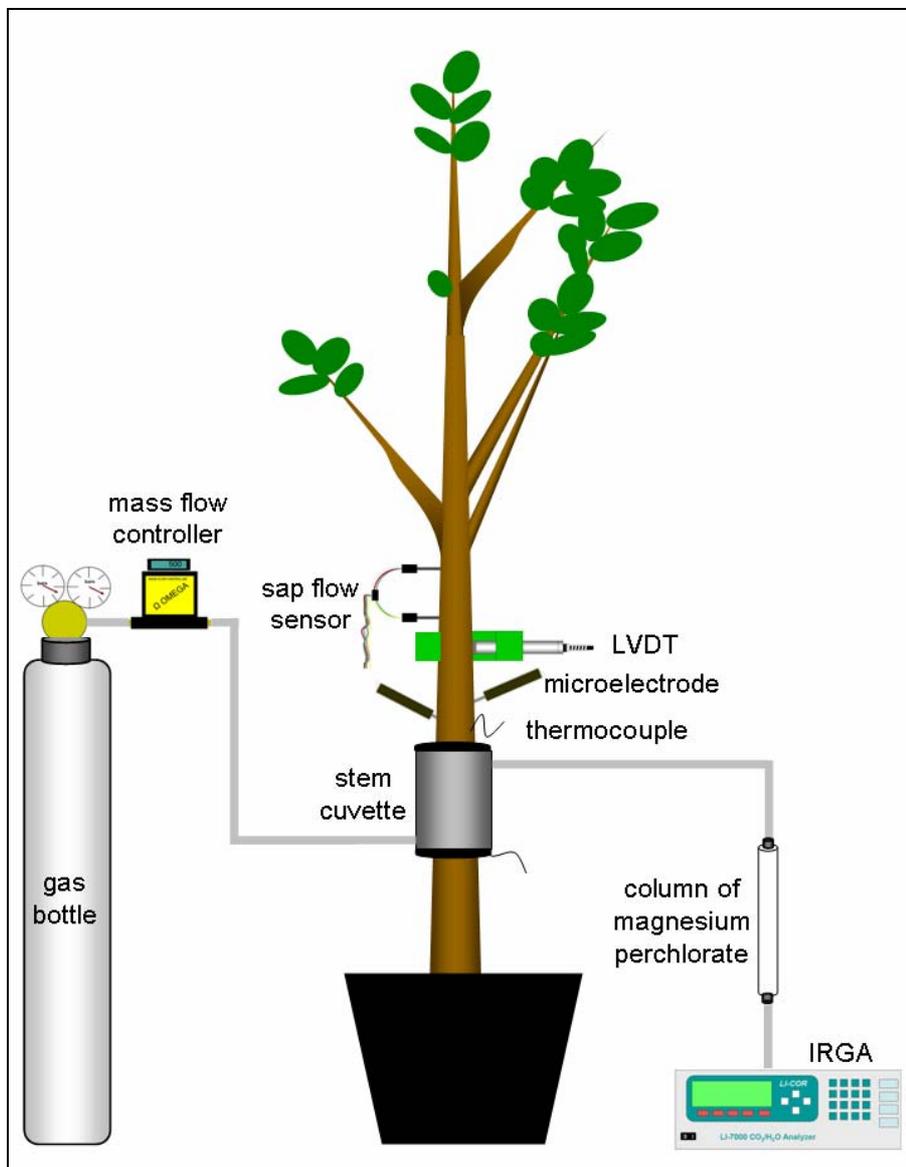


Figure 6.1: Schematic overview of the experimental setup in the growth chamber at the University of Georgia. Specifications about the sensors and devices are given in the text.

CO₂ concentration ([CO₂]; %) in the xylem was measured with CO₂ microelectrodes (Model MI-720, Microelectrodes Inc, Bedford, NH, USA) as described in McGuire and Teskey (2002, 2004). Two CO₂ microelectrodes were placed in the xylem 20 and 40 mm above the cuvette using brass tubes fitted into holes of 10 mm deep and 4 mm diameter. The microelectrodes were inserted into the tubes and flexible putty adhesive was used to seal the junction between the tube and stem, and Parafilm M film (American National Can, Menasha, WI, USA) to seal the junction between the stem and sensor. The microelectrodes measured internal [CO₂] in air in a small headspace in equilibrium with the xylem sap. Data from two microelectrodes were averaged for each tree. For calculating the resistance to radial CO₂ diffusion (Equation 6.2), the data collected from the CO₂ microelectrodes was converted into mole concentration. Stem temperature (T_{st}) was measured with a thermocouple inserted 5 mm into the stem near the top of the cuvette.

Stem sap velocity (v_s ; cm h⁻¹) was measured at 8 mm depth with a thermal dissipation probe (Model TDP-30, Dynamax Inc., Houston, TX, USA) (Granier 1985, 1987). The sensor was thermally insulated with open-cell foam and several layers of aluminium foil.

Stem diameter variation (D ; mm) was measured with a linear variable displacement transducer (LVDT; LBB 375-PA-100 and transducer bridge 8C-35, Schaevitz, VA, USA). The LVDT was fixed on the stem with a custom-made stainless steel holder and measured whole-stem (i.e., over-bark) diameter variations and, hence, the integrated effect of changes in water reserves of the living stem tissues (bark, phloem, cambium) and the xylem.

All sensors and the cuvette were covered with a sheet of reflective bubble insulation (Reflectix, Markleville, IN, USA) to exclude radiation and thus avoiding cortical and wood photosynthesis. Measurements were made every 10 s and recorded every 5 min with a data logger (Model CR23X, Campbell Scientific Inc, Logan, UT, USA). We were limited by available equipment to measuring one tree at a time.

6.2.3 Experiments

Three cottonwood trees were subjected to a direct manipulation of [CO₂] of xylem sap in order to investigate the relationship between E_{CO_2} and xylem [CO₂]. A 7-mm diameter hole was drilled 10 mm into the xylem 0.1 m above the soil and a brass tube (7 mm diameter × 35 mm length) was tightly fitted into the hole to a depth of 8 mm. To ensure good seal flexible putty adhesive was used at the junction of the brass tube and the tree. Tygon tubing was used to connect the brass tube to a plastic reservoir filled with water that had been enriched with CO₂ by bubbling air at 10% CO₂ from a compressed gas cylinder (Figure 6.2). Xylem

tension pulled this water into the tree, where it mixed with the xylem sap. During these manipulation experiments, air temperature (T_a) was kept constant at 22.3 ± 0.1 °C. After at least 8 h of direct manipulation in the light, the lights in the growth chamber were switched off. In all trees, E_{CO_2} , xylem $[CO_2]$, and T_{st} were measured simultaneously.

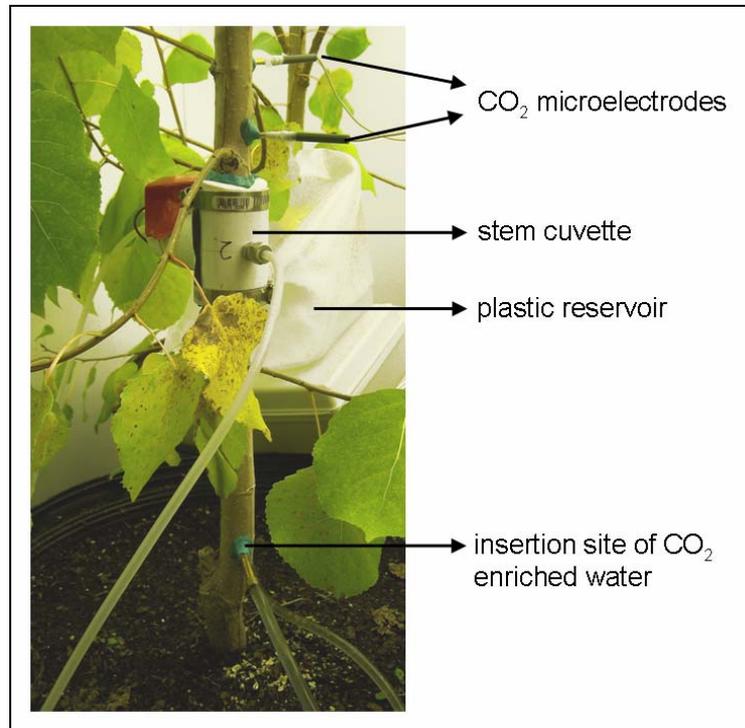


Figure 6.2: Setup for the sap manipulation experiment. After installation the sensors and cuvette were covered with reflective bubble insulation to prevent corticular and wood photosynthesis.

Additionally, the relationship between E_{CO_2} and xylem $[CO_2]$ was further investigated in an intact (non-manipulated) cottonwood tree over a 24-h period (14 h light, 10 h dark). T_a in the stem cuvette was constant (25.0 ± 0.1 °C). In this tree, v_s and D were measured in addition to E_{CO_2} , xylem $[CO_2]$, and T_{st} .

For all experiments, mean PAR during the light period was approximately $700 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the top of the tree. Vapour pressure deficit of the air fluctuated between 0.37 ± 0.05 kPa during the dark period and 2.27 ± 0.21 kPa during the light period.

6.3 Results

6.3.1 Manipulation of xylem CO₂ concentration

In all trees, sap manipulation at the stem base was effective in changing xylem $[CO_2]$ at approximately 0.5 m above soil level (Figure 6.3), indicating that CO₂ was transported with the flowing xylem sap. We observed a consistent gradual

increase in measured xylem [CO₂] in all stems, but the starting point of increase differed among the trees. While xylem [CO₂] immediately began to increase in Tree 1 and 2 when water enriched with CO₂ was infused into the xylem, a delay of approximately 5 h was observed in Tree 3. Besides an increase in xylem [CO₂], infusion with 10% CO₂-enriched water into the xylem also produced a corresponding change in E_{CO_2} (Figure 6.3). The increase in E_{CO_2} either coincided with the increase in xylem [CO₂] in Tree 2 and 3 or occurred after 2 h in Tree 1. The parallel change in E_{CO_2} and xylem [CO₂] indicates that CO₂ transported in the xylem could radially diffuse and affect E_{CO_2} measurements. When lights were switched off, a rapid and parallel change in xylem [CO₂] and E_{CO_2} was observed (Figure 6.3).

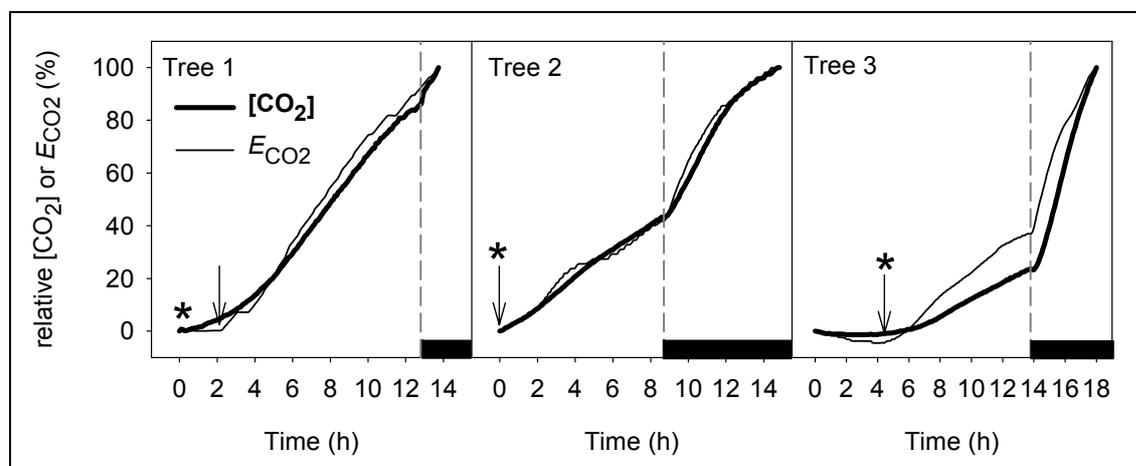


Figure 6.3: Typical changes in measured xylem CO₂ concentrations ([CO₂]) and stem CO₂ efflux rates (E_{CO_2}) following infusion (at time zero) with 10% CO₂-enriched water into the xylem of three trees of a *Populus* clone. Values are expressed relative to maximum values measured during the manipulation experiment. Vertical dashed lines represent the transition from light to dark conditions. The arrows indicate the starting point of increase in E_{CO_2} and the stars indicate the starting point of increase in [CO₂].

6.3.2 Relationship between CO₂ efflux and xylem CO₂ concentration

Among the individual trees, substantial differences in xylem [CO₂] were evident (Figure 6.4). During the light period, xylem [CO₂] ranged from 5.9 to 12.3% in Tree 1, from 7.5 to 14.7% in Tree 2 and from 5.9 to 9.2% in Tree 3. Xylem [CO₂] was much higher than that of ambient air. In all trees, stem E_{CO_2} was closely correlated with xylem [CO₂]. Plotting the daytime data for each individual tree revealed clear linear relationships, with high R^2 (Table 6.1). Stem E_{CO_2} ranged from 331 to 701 $\mu\text{mol m}^{-3} \text{s}^{-1}$ in Tree 1, from 231 to 368 $\mu\text{mol m}^{-3} \text{s}^{-1}$ in Tree 2 and from 418 to 736 $\mu\text{mol m}^{-3} \text{s}^{-1}$ in Tree 3.

The slope of the straight line relating E_{CO_2} to xylem [CO₂] substantially differed among the trees (Figure 6.4). Resistance to radial CO₂ diffusion was calculated as the inverse of the slope of the linear relationship between E_{CO_2} and [CO₂],

whereby E_{CO_2} was expressed per stem surface area and $[\text{CO}_2]$ as the difference between xylem and air (Equation 6.4, Figure 6.4, Table 6.1). The resistance appeared to be tree-specific and differed 6-fold among the trees (Table 6.1). A high resistance to radial CO_2 diffusion resulted in a large increase in xylem $[\text{CO}_2]$, accompanied by a limited increase in E_{CO_2} (Tree 2, Figure 6.4). On the contrary, in a stem with a low barrier to CO_2 diffusion, CO_2 rapidly escaped to the atmosphere (steep increase in E_{CO_2}) rather than accumulating in the xylem (limited increase in xylem $[\text{CO}_2]$) (Tree 3, Figure 6.4).

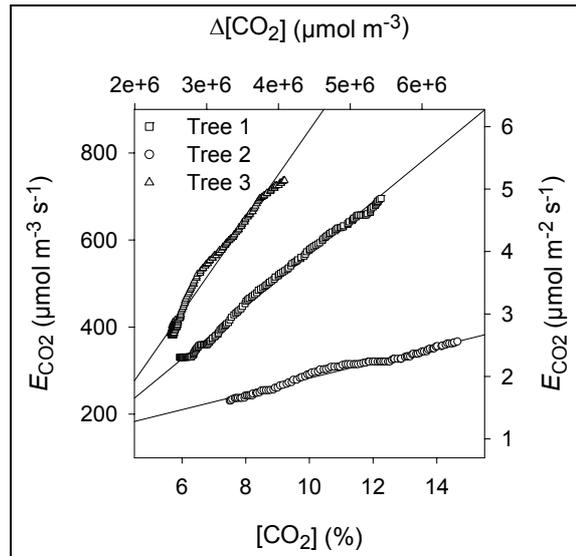


Figure 6.4: Linear relationships between stem CO_2 efflux rates (E_{CO_2}) and xylem CO_2 concentrations ($[\text{CO}_2]$) during the light period in three trees of a *Populus* clone after direct manipulation of xylem $[\text{CO}_2]$. E_{CO_2} is expressed per sapwood volume (left axis) or per stem surface area (right axis). $[\text{CO}_2]$ is expressed in percentage (bottom axis) or as the difference between xylem and air $[\text{CO}_2]$ in mole concentration (top axis). Lines are the linear regressions between E_{CO_2} and $\Delta[\text{CO}_2]$. Regression equations of E_{CO_2} versus $\Delta[\text{CO}_2]$ are listed in Table 6.1.

Table 6.1: Coefficients of the linear relationship between stem CO_2 efflux rates (E_{CO_2} , in $\mu\text{mol m}^{-2} \text{s}^{-1}$) and CO_2 concentrations ($\Delta[\text{CO}_2]$, in $\mu\text{mol m}^{-3}$) in three trees of a *Populus* clone. Linear regression lines are shown in Figure 6.4. Resistance to radial CO_2 diffusion is calculated as the inverse of the slope of the linear regression. R^2 is the coefficient of determination. Standard errors are given in parentheses.

Tree	Slope	Intercept	R^2	Resistance (s m^{-1})
Light period				
1	9.405e-7 (5.212e-9)	-0.222 (0.0207)	0.99	1.063e+06
2	2.816e-7 (4.295e-9)	0.721 (0.0217)	0.98	3.552e+06
3	1.636e-6 (1.720e-8)	-1.326 (0.0522)	0.98	6.112e+05
Dark period				
1	5.576e-7 (6.144e-9)	1.784 (8.008e-2)	0.99	1.794e+06
2	2.763e-7 (1.930e-9)	1.018 (1.93e-2)	0.99	3.740e+06
3	5.99e-7 (5.980e-9)	3.559 (9.539e-2)	0.98	1.669e+06

The resistance to radial CO₂ diffusion also affected the timing of detection of a change in xylem [CO₂] and E_{CO_2} when CO₂-enriched water was infused into the xylem. For instance, when the resistance to radial CO₂ diffusion was small, the build-up in xylem [CO₂] and the increase in E_{CO_2} was delayed (Tree 3, Figure 6.3), because CO₂ could easily escape out of the stem before it reached the cuvette and the microelectrodes at 0.5 m above soil level. On the contrary, when the resistance was large, xylem [CO₂] and E_{CO_2} increased immediately following the infusion (Tree 2, Figure 6.3).

Switching off the lights coincided with a rapid increase in both xylem [CO₂] and E_{CO_2} of all trees (Figure 6.3). Xylem [CO₂] increased from 12.3 to 13.3% in Tree 1, from 14.7 to 24.2% in Tree 2 and from 9.2 to 20.2% in Tree 3. Again, E_{CO_2} closely corresponded with xylem [CO₂] and increased from 701 to 1730 $\mu\text{mol m}^{-3} \text{s}^{-1}$, from 368 to 558 $\mu\text{mol m}^{-3} \text{s}^{-1}$ in Tree 2 and from 737 to 1280 $\mu\text{mol m}^{-3} \text{s}^{-1}$ in Tree 3. Plotting the dark period data revealed a positive linear relationship between E_{CO_2} and xylem [CO₂], with a weaker slope compared to that observed in the light period (Table 6.1, Figure 6.5), indicating that the resistance to radial CO₂ diffusion was affected by the transition from light to dark conditions, at least for trees with relatively small daytime resistances.

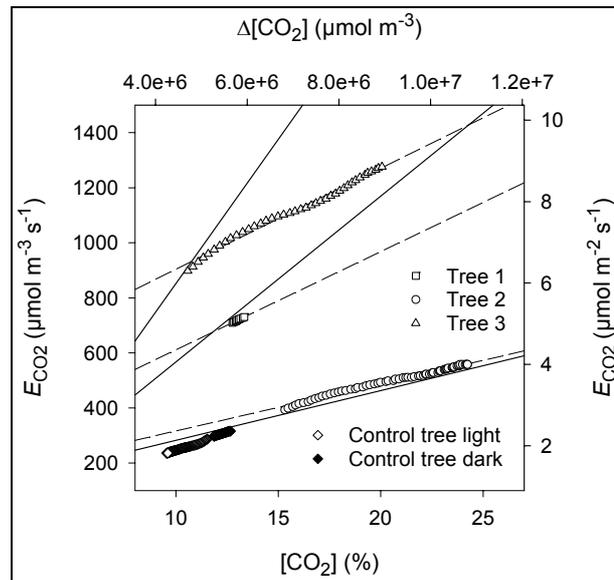


Figure 6.5: Linear relationships between stem CO₂ efflux rates (E_{CO_2}) and xylem CO₂ concentrations ($[CO_2]$) during the dark period for the three manipulated trees and during the light and dark period for the intact (non-manipulated) tree of a *Populus* clone.] E_{CO_2} is expressed per sapwood volume (left axis) or per stem surface area (right axis). $[CO_2]$ is expressed in percentage (bottom axis) or as the difference between xylem and air $[CO_2]$ in mole concentration (top axis). Solid lines are the linear regressions between E_{CO_2} and $\Delta[CO_2]$ during the light period (Figure 6.4), whereas dashed lines are the linear regressions during the dark period. Regression equations of E_{CO_2} versus $\Delta[CO_2]$ are listed in Table 6.1.

In the intact (non-manipulated) tree, xylem $[\text{CO}_2]$ changed during the 24-h period, from low values during the light period to high values during the dark (Figure 6.6b). Xylem CO_2 typically ranged from 9.5 to 12.7%, which again is much higher than that of ambient air. E_{CO_2} correlated well with xylem $[\text{CO}_2]$ ($R^2 = 0.91$). Similar to the manipulation experiments, a clear linear relationship was found between E_{CO_2} and xylem $[\text{CO}_2]$ during both light and dark period (Figure 6.5). Compared to the manipulated trees, the intact tree appeared to have a high resistance to radial CO_2 diffusion, which resulted in high xylem $[\text{CO}_2]$ (Figure 6.6b) and a limited response of E_{CO_2} to changes in $[\text{CO}_2]$ (Figure 6.5).

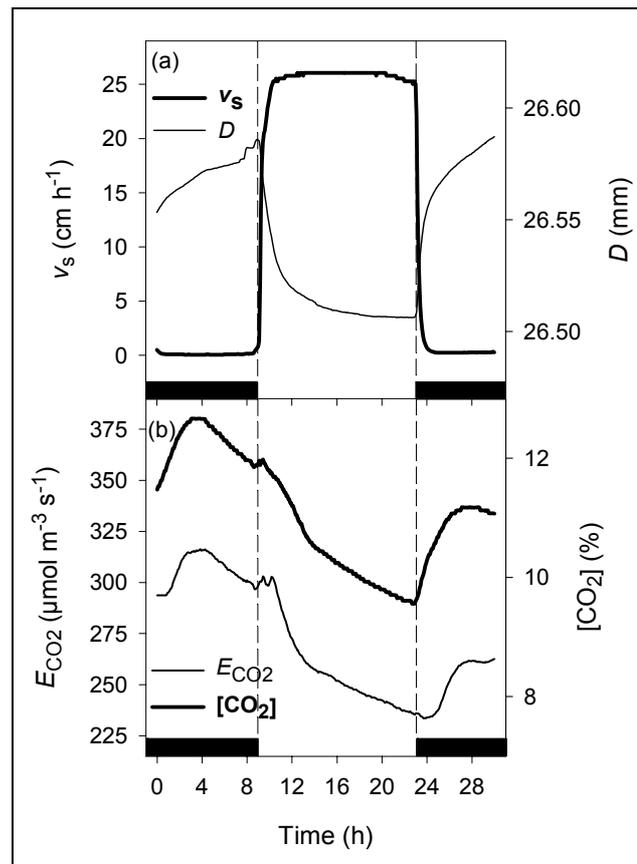


Figure 6.6: Diurnal pattern of (a) sap velocity (v_s) and stem diameter (D), and (b) stem CO_2 efflux rate (E_{CO_2}) and xylem CO_2 concentration ($[\text{CO}_2]$) measured in the intact (non-manipulated) tree of a *Populus* clone under controlled conditions. The air temperature was kept constant at 25.0 ± 0.1 °C. Vertical dashed lines correspond to the beginning and end of the light period.

6.3.3 Diurnal pattern of CO_2 efflux and xylem CO_2 concentration

In the intact tree, neither E_{CO_2} nor xylem $[\text{CO}_2]$ were related to stem or air temperature (which were kept constant), indicating that other factors affected the diurnal patterns of E_{CO_2} and xylem $[\text{CO}_2]$. When v_s was at a maximum during the light period, $[\text{CO}_2]$ gradually tended towards a minimum (Figure 6.6), suggesting that flowing sap diluted $[\text{CO}_2]$ in the xylem during the light period. In addition, E_{CO_2} and xylem $[\text{CO}_2]$ exhibited a variation during the light period that was well

correlated with the change in D ($R^2 = 0.77$ and 0.72 for E_{CO_2} and xylem $[\text{CO}_2]$, respectively). During the dark period, D increased gradually as did E_{CO_2} and xylem $[\text{CO}_2]$, but E_{CO_2} and xylem $[\text{CO}_2]$ peaked earlier than D , resulting in poorer coefficients of determination during the dark period ($R^2 = 0.37$ and 0.46 for E_{CO_2} and xylem $[\text{CO}_2]$, respectively). Maximum D occurred at the end of the dark period. Onset of the light period caused a sharp decrease in D , followed by a decrease in xylem $[\text{CO}_2]$ and E_{CO_2} .

6.4 Discussion

6.4.1 Xylem CO₂ concentrations

Xylem $[\text{CO}_2]$ measured in this study ranged from 9.5 to 12.7% in the intact tree and from 5.9 to 24.2% in the manipulated trees and was two orders of magnitude greater than atmospheric $[\text{CO}_2]$. These observations are consistent with previous reports of stem $[\text{CO}_2]$, ranging from less than 1% to over 26% (Bushong 1907, MacDougal and Working 1933, Chase 1934, Jensen 1967, Eklund 1990, 1993, Hari et al. 1991, Levy et al. 1999, Teskey and McGuire 2002). It has been well-recognized that such high values, relative to atmospheric $[\text{CO}_2]$, indicate that there are significant barriers to radial diffusion of CO₂ from the xylem to the atmosphere (Eklund and Lavigne 1995). Respiratory CO₂, evolved inside the stem and moving outward to the atmosphere, must overcome the resistance of the xylem (Sorz and Hietz 2006), the cambium (Hook et al. 1972, Kramer and Kozlowski 1979) and the bark layers (Lendzian 2006). This restriction to the radial movement of CO₂ might substantially affect estimates of stem respiration rates based on E_{CO_2} , because it has a critical impact on the rate of diffusion of CO₂ from stem to atmosphere. Gansert and Burgdorf (2005) suggested that measured E_{CO_2} from the stem may originate physico-chemically from a strong diffusion gradient, rather than from actual respiration of living tissues. The resistance to CO₂ diffusion might therefore introduce errors in efflux-based estimates of woody tissue respiration rates, because the basic assumption was that all CO₂ had recently evolved from local respiring cells (Teskey and McGuire 2002).

6.4.2 Between-tree variability in diffusion resistance

The resistance determines the balance between the amount of CO₂ retained in the xylem ($[\text{CO}_2]$) versus that which escapes to the atmosphere (E_{CO_2}). We observed substantial differences in E_{CO_2} between individual trees of the same clone (Figure 6.4). The possibility that differences in E_{CO_2} among replications were due to variations in environmental factors was excluded, because all experiments were conducted under controlled conditions with constant temperature and radiation level. Direct effects of temperature on the respiration

rate, the diffusion coefficient of CO₂ (Fick's law) and the solubility of CO₂ were therefore of no relevance. More likely, the difference in E_{CO_2} among the trees is directly attributable to the difference in the resistance to radial CO₂ diffusion.

The different slopes of the straight lines relating E_{CO_2} to xylem [CO₂] (Figure 6.4, Table 6.1) demonstrate that the resistance to radial CO₂ diffusion may substantially differ among trees. Similarly, Teskey and McGuire (2002) found that when water enriched with 10% CO₂ was pushed through branch segments of *Liriodendron tulipifera* L. and *Quercus alba* L., E_{CO_2} and xylem [CO₂] changed proportionally, but calculated resistances to radial CO₂ diffusion varied between 2.91e+6 and 6.53e+6 s m⁻¹ (i.e., 2-fold) in *Liriodendron* and between 7.30e+5 and 3.84e+6 s m⁻¹ (i.e., 5-fold) in *Quercus*, showing a high variability in diffusion resistance between branches. In our study, diffusion resistances varied 6-fold between the individual trees (Table 6.1). We expected more consistency in resistance among the trees due to the identical genetic material used in this study. However, several plausible reasons can be put forward explaining why the resistances were so variable in our individuals. It is possible that the differences were caused by the visually observed differences in bark properties between the trees. For instance, the bark of Tree 2 was rougher (i.e., bark with wart-like structures) than the other two individuals, which apparently resulted in a higher resistance. Although no measurements of stem tissue properties were made in this study, it has been documented (1) that xylem permeability is dependent on the arrangement of cell walls, the structure of cell walls, and/or pit membranes (Sorz and Hietz 2006), (2) that permeability of the cambium is determined by the abundance of intercellular spaces (Hook et al. 1972) and (3) that bark permeability depends on its chemical constituents (lignins, suberins, lipids and waxes) (Schönherr 1982) and its abundance of lenticels, cracks and wounds (Grosse 1997, Langenfeld-Heyser 1997). Schönherr and Ziegler (1980) found that periderm with lenticels is 26 to 53 times more permeable than periderm without lenticels. Furthermore, it has been shown by Klasnja et al. (2003) that significant intra-clonal differences exist in woody tissue properties (chemical composition, fiber length and basic wood density) between individuals of *Populus deltoides* clones. The large variability in resistance of stem tissues may also be caused by differences in the stem water content. A higher stem water content most likely increases the diffusion resistance because the diffusion coefficient of CO₂ in water is 10⁴ times slower in water than in air (Nobel 1999). Tissue water content depends on v_s , so that differences in v_s may explain between-tree variability in diffusion resistance of stems.

Differences in resistances among tree stems result in different portions of respiratory CO₂ retained in the xylem. High resistances of stem tissues imply that a large amount of respiratory CO₂ of the stem will not escape to the atmosphere,

resulting in large xylem [CO₂]. Hence, in trees with high resistances, the amount of CO₂ that can be transported along with the transpiration stream is higher than in trees with small resistances. Therefore, estimating the actual stem respiration rate based on measurements of E_{CO_2} in tree stems with large resistances will introduce larger errors compared to tree stems with small resistances. Another implication is that in tree stems with small resistances, the contribution of respired CO₂ originating from lower parts (soil, roots, lower stem parts) to stem E_{CO_2} will be smaller than in trees with large resistances, because a large part of respiratory CO₂ escapes immediately to the atmosphere instead of being dissolved in the sap and being transported with the transpiration stream. Differences in resistance among trees could be an important overlooked cause for the high variability in stem E_{CO_2} among trees and for the absence of consistent relationships between stem E_{CO_2} and environmental factors, tissue sizes, tree ages or species.

6.4.3 Diurnal pattern of CO₂ efflux and xylem CO₂ concentration

Transition from light to dark conditions affected the resistance to radial CO₂ diffusion (Table 6.1, Figure 6.5). When lights were switched off, a rapid increase in xylem [CO₂] was observed, which proportionally affected E_{CO_2} (Figures 6.1 and 6.4b). The higher resistance in the dark period compared to the light is likely due to an increased tissue water content during the dark period (zero sap flow conditions), which slows down radial diffusion (diffusion of CO₂ in water is approximately 10⁴ times slower in water than in air).

Patterns of xylem [CO₂] and E_{CO_2} were not related to T_a or T_{st} . Although the effects of temperature on rates of respiration at the cellular level are well established (Amthor 1989), our data indicate that stem E_{CO_2} cannot be adequately predicted by temperature alone. Because temperature was kept constant, the rapid increase in xylem [CO₂] and E_{CO_2} at the onset of dark might be related to actual respiratory CO₂ caused by enhanced respiration due to an improved water status and higher turgor pressure in the absence of sap flow (Daudet et al. 2005, Saveyn et al. 2007a). The shrinkage and swelling pattern of D of the intact (non-manipulated) tree (Figure 6.6a) indicates that there was a diurnal change in stem water content as a result of sap flow (Steppe et al. 2006). The minimum D occurred just before the end of the light period, presumably due to depletion of internal water reserves, and the maximum D occurred at the end of the dark period, when optimal tissue water content or turgor was restored. The similarity between the daily variation of xylem [CO₂] and E_{CO_2} on the one hand and D on the other hand (Figures 6.6a and 6.6b) supports the assumption that higher growth and maintenance metabolism might have occurred during the dark, in relation to water status improvements (Daudet et al. 2005, Saveyn et al. 2007a). It is, however, not clear why E_{CO_2} and xylem [CO₂] peaked before D during the dark period (Figure 6.6).

Another possible explanation for the daytime depressions in E_{CO_2} and xylem $[\text{CO}_2]$ is that during the light period sap flow diluted $[\text{CO}_2]$ in the xylem because it introduced sap coming from the soil at much lower $[\text{CO}_2]$ in the stem. During the dark, when sap flow was zero, xylem $[\text{CO}_2]$ increased because the dilution ceased and CO_2 produced by the living stem tissues was further added to the xylem sap. With the available data we could not distinguish between these two effects of sap flow (reduced stem water status and dilution of xylem sap $[\text{CO}_2]$) on stem E_{CO_2} and xylem $[\text{CO}_2]$.

In the manipulated trees, it was remarkable that the xylem $[\text{CO}_2]$ measured in the stems was often higher than the $[\text{CO}_2]$ in the treatment water. Dark conditions seemed to amplify this effect (Figure 6.5). Teskey and McGuire (2005) sometimes observed higher xylem $[\text{CO}_2]$ in *Liquidambar styraciflua* compared to the initial $[\text{CO}_2]$ of the water infused into the xylem. A plausible explanation would be that injured but functioning cells and uninjured neighbouring cells may have increased their rate of respiration as a direct response to damage caused by drilling the infusion hole. However, wound respiration occurred 0.4 m below the cuvette and the respiratory CO_2 produced at the site of drilling could not possibly be transported upward in the sap to the sites of measurement during zero sap flow conditions in the dark. Also, a direct effect of vertical CO_2 diffusion from sites with high $[\text{CO}_2]$ (at the site of drilling) to sites with low $[\text{CO}_2]$ (at the site of measurement) according to Fick's law is unlikely, because it takes on average 40 min for CO_2 with a diffusion coefficient of $1.6 \times 10^{-5} \text{ m}^2 \text{ s}^{-1}$ to diffuse 0.4 m in air (Nobel 1999). Given the fact that stems contain a substantial amount of water and the diffusion coefficient in water is approximately 10^4 times smaller than that in air (Nobel 1999), CO_2 could not ascend the stem by diffusion at a rate sufficient to explain the direct increase in E_{CO_2} and $[\text{CO}_2]$ at 0.5 m above soil level at the onset of the dark period. There seems, therefore, no reason to assume that the rapid increase in xylem $[\text{CO}_2]$ and E_{CO_2} was due to wound respiration. From our data we cannot reach any firm conclusions on the real reason why manipulated trees showed a more pronounced increase in E_{CO_2} and $[\text{CO}_2]$ at the onset of dark in comparison to the intact, non-manipulated tree.

6.5 Conclusions

Artificial introduction of CO_2 enriched water at the stem base lead to a change in xylem $[\text{CO}_2]$ at 0.5 m above soil level, indicating that CO_2 was transported with the xylem sap. The increase in xylem $[\text{CO}_2]$ resulted in a rise of stem E_{CO_2} . The degree of response of E_{CO_2} to changes in xylem $[\text{CO}_2]$ is a measure for the resistance of the stem to radial CO_2 diffusion. Genetically identical tree stems were found to possess substantially different resistances. High resistances imply that a large amount of respiratory CO_2 will not escape to the atmosphere but will

be retained in the xylem, leading to the conclusion that the stem respire at a substantially higher rate than is accounted for by E_{CO_2} measurements. Differences in resistance to CO₂ diffusion also imply that the total quantity of CO₂ that fluxes from the stem to the atmosphere will consist of varying proportions of CO₂ contributed by local respiring cells and transported CO₂. The variability of the resistance to radial CO₂ diffusion, which might be expected to change within a tree across the growing season and with tissue age, might be an overlooked factor explaining the high variation reported in E_{CO_2} of branches and stems among trees.

Chapter 7

Stem respiration and CO₂ efflux of young Populus deltoides trees in relation to temperature and xylem CO₂ concentration

Adapted from : Saveyn A, Steppe K, McGuire MA, Lemeur R and Teskey RO (2007) Stem respiration and CO₂ efflux of young *Populus deltoides* trees in relation to temperature and xylem CO₂ concentration. *Oecologia* (accepted)

Abstract

Oxidative respiration is strongly temperature-driven. However, in woody stems, efflux of CO₂ to the atmosphere (E_{CO_2}), commonly used to estimate the rate of respiration (R_S), and stem temperature (T_{st}) have often been poorly correlated, which we hypothesized was due to transport of respired CO₂ in xylem sap, especially under high rates of sap flow (f_s). To test this, we measured E_{CO_2} , T_{st} , f_s and xylem sap CO₂ concentrations ($[\text{CO}_2^*]$) in three-year-old *Populus deltoides* trees under different weather conditions (sunny and rainy days) in autumn. We also calculated R_S by mass balance as the sum of both outward and internal CO₂ fluxes and hypothesized that R_S would correlate better with T_{st} than E_{CO_2} . We found that E_{CO_2} sometimes correlated well with T_{st} , but not on sunny mornings and afternoons or on rainy days. When the temperature effect on E_{CO_2} was accounted for, a clear positive relationship between E_{CO_2} and xylem $[\text{CO}_2^*]$ was found. $[\text{CO}_2^*]$ varied diurnally and increased substantially at night and during periods of rain. Changes in $[\text{CO}_2^*]$ were related to changes in f_s but not T_{st} . We conclude that changes in both respiration and internal CO₂ transport altered E_{CO_2} . The dominant component flux of R_S was E_{CO_2} . However, on a 24-h basis, the internal transport flux represented 9 – 18% and 3 – 7% of R_S on sunny and rainy days, respectively, indicating that the contribution of stem respiration to forest carbon balance may be larger than previously estimated based on E_{CO_2} measurements. Unexpectedly, the relationship between R_S and T_{st} was sometimes weak in two of the three trees. We conclude that in addition to temperature, other factors such as water deficits or substrate availability exert

control on the rate of stem respiration so that simple temperature functions are not sufficient to predict stem respiration.

7.1 Introduction

CO₂ efflux rates from stems (E_{CO_2}) are expected to be closely related to the respiration rates of the living cells in stem tissues (phloem, cambium and xylem parenchyma). Since respiration consists of enzymatic reactions, it is known to be highly temperature dependent (Amthor 1989). Strong relationships between E_{CO_2} and temperature (T) have been reported (e.g., Levy and Jarvis 1998, Damesin et al. 2002, Gansert et al. 2002). However, the relationship between E_{CO_2} and T has also been reported to be unclear (Edwards and McLaughlin 1978, Kakubari 1988, Kaipainen et al. 1998). These observations gave rise to the idea that E_{CO_2} is not just a reflection of the actual respiration rate (R_S) of the living cells in the woody tissues. It has been suggested that sap flow (f_s) might influence E_{CO_2} . Given the high solubility of CO₂ in water, respired CO₂ at stem level can dissolve in the xylem sap and may be carried upward with the transpiration stream instead of diffusing radially through the bark (Negisi 1979, Martin et al. 1994). Additionally, CO₂ originating from root tissue or soil microbial respiration can dissolve in xylem sap, be carried upward, and be released from the stem remote from the site of respiration (Levy et al. 1999, Teskey and McGuire 2007). Hence, E_{CO_2} may under- or overestimate the actual respiration rate of the stem tissue due to transport of CO₂ by the transpiration stream. Several studies have established the relationship between f_s and E_{CO_2} (Negisi 1979, Martin et al. 1994, Levy et al. 1999, Edwards and Wullschlegler 2000, Bowman et al. 2005, Gansert and Burgdorf 2005, Maier and Clinton 2006). However, f_s measurements do not provide direct information about CO₂ transport inside the stem.

Recently, McGuire and Teskey (2004) proposed a mass balance approach for estimating the respiration rate (R_S) of a stem segment that accounts for both external (i.e., E_{CO_2}) and internal fluxes of CO₂. Internal fluxes comprise the CO₂ transport by flowing sap (F_T) and the transient changes in the storage of CO₂ within the stem (ΔS). To calculate the CO₂ transport rate, f_s and the xylem CO₂ concentration ($[\text{CO}_2]$) above and below the stem segment need to be measured. Recently, methods have been developed to directly measure the dynamics of xylem $[\text{CO}_2]$ *in situ* (McGuire and Teskey 2002, 2004, Teskey and McGuire 2007). A few reports have established the relationship between xylem $[\text{CO}_2]$ and E_{CO_2} (Teskey and McGuire 2002, 2005, 2007, Maier and Clinton 2006, McGuire and Teskey 2007, Saveyn et al. 2006, 2007b). However, in very few studies (McGuire and Teskey 2004, Bowman et al. 2005, Teskey and McGuire 2007) R_S has actually been calculated. Because internal fluxes are accounted for, it is expected that calculated R_S would correlate better with T_{st} than E_{CO_2} . However,

as far as we know, the relationship between calculated R_S based on mass balance and T_{st} has never been investigated.

The first objective of this work was to elucidate the driving variables of E_{CO_2} in a clone of *Populus deltoides* during autumn. The effects of both T_{st} and xylem $[CO_2]$ were analyzed under different weather conditions (sunny and rainy days). The second objective was to calculate R_S by mass balance and to examine the relationship between T_{st} and R_S . Because calculated R_S accounts for both external and internal fluxes of CO_2 , and represents the actual respiration rate of the stem tissue, it was hypothesized that the correlation between R_S and T_{st} would be better than the correlation between E_{CO_2} and T_{st} .

7.2 Materials and methods

7.2.1 Plant material and experimental conditions

Field experiments were conducted during autumn in a nursery at Whitehall Forest, an experimental forest of the University of Georgia near Athens, Georgia, from 13 October to 1 November 2006 (day 286 - 305). Measurements were made on three three-year-old trees of an eastern cottonwood (*Populus deltoides* Bartr. ex Marsh.) clone (Clone 98, Meadwestvaco Corp, Paducah, KY, USA). Stem diameters were 8.9, 7.7 and 7.8 cm at 0.5 m above ground for Tree 1, 2 and 3, respectively. Due to limited available equipment, only one tree at a time was measured. Measurement periods were day 286 - 291 for Tree 1, day 292 - 298 for Tree 2 and day 299 - 305 for Tree 3.

7.2.2 Measurements

Stem CO_2 efflux (E_{CO_2}) was measured in the field, in a fan-stirred 8.5 cm-long cylindrical Lexan cuvette that completely surrounded the stem at 0.5 m above ground. The cuvette was secured to the stem at both ends with closed cell foam gaskets and hose clamps. To provide a gas-tight seal, glazing putty was used to cover the gaskets. The cuvette and the stem parts above and below the cuvette were covered with aluminium foil to prevent cortical and wood photosynthesis (Figure 7.1). Compressed air at near-ambient $[CO_2]$ was supplied to the cuvette at 0.5 l min^{-1} using a mass flow controller (model FMA 5514, Omega Engineering, Stamford, CT, USA). Air leaving the cuvette was dried over a column of magnesium perchlorate, and its CO_2 concentration was measured with an infrared gas analyser (IRGA) (Model 7000, Li-Cor Inc., Lincoln, NE, USA). The IRGA was operated in open configuration and E_{CO_2} ($\mu\text{mol } CO_2 \text{ m}^{-3} \text{ s}^{-1}$) was calculated as:

$$E_{CO_2} = (f_a/V) \times \Delta[CO_2] \quad (7.1)$$

where f_a is the flow rate of air through the cuvette ($3.7 \times 10^{-4} \text{ mol s}^{-1}$), V is the sapwood volume of the stem segment (m^3) and $\Delta[\text{CO}_2]$ is the difference between $[\text{CO}_2]$ of air flowing into and out of the cuvette ($\mu\text{mol mol}^{-1}$). To determine sapwood volume, stem diameter was measured with a diameter tape at the top and bottom of the cuvette, and bark thickness was measured from the increment cores that were taken for pH measurements (described below). The trees contained no heartwood.

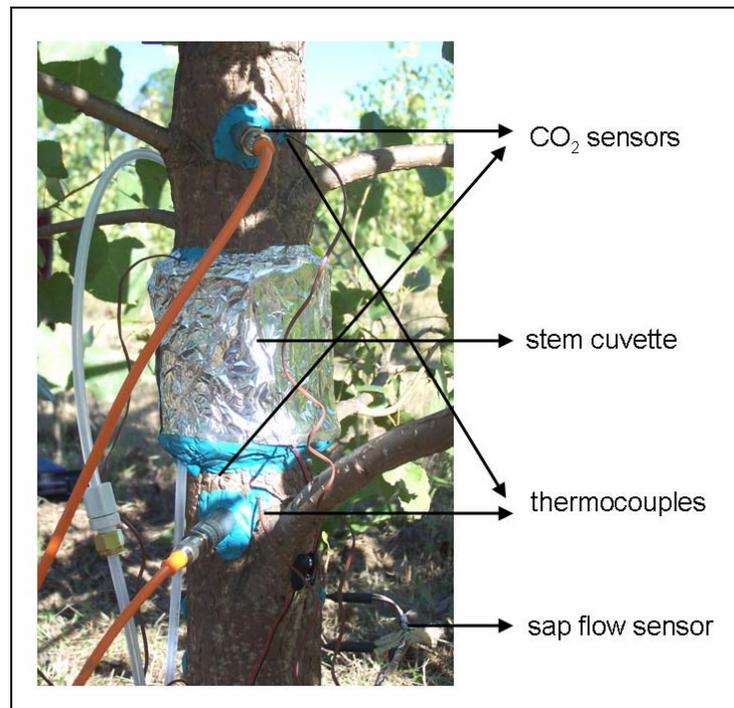


Figure 7.1: Setup for the experiment. Specifications about the sensors and cuvette are given in the text. After installation the sensors and cuvette were covered with reflective bubble insulation to prevent cortical and wood photosynthesis.

Xylem CO_2 concentration ($[\text{CO}_2]$, %) was measured *in situ* with two solid state non-dispersive infrared (NDIR) CO_2 sensors (Model GMM221, Vaisala, Helsinki, Finland) inserted in the stems in holes of 50 mm length and 19 mm diameter (Figure 7.1). The holes were drilled 50 mm above and below the cuvette. Flexible putty adhesive (Qubitac, Qubit Systems, Kingston, Ontario, Canada) was used to provide a gas-tight seal at the junction of the sensor and stem. The CO_2 sensors measured $[\text{CO}_2]$ of the gas in the headspace of the holes, which is in equilibrium with the xylem sap (Hari et al. 1991, Levy et al. 1999). Gas concentration (%) was converted to total dissolved carbon ($[\text{CO}_2^*]$, $\mu\text{mol l}^{-1}$) using temperature-dependent Henry's solubility and dissociation constants for CO_2 in water (Butler 1991, Stumm and Morgan 1996, McGuire and Teskey 2002). For this conversion, stem temperature (T_{st}) and pH of the xylem sap must be known. T_{st} was measured with 2 copper-constantan thermocouples inserted 50 mm deep in the stem near the CO_2 sensors (Figure 7.1). Two methods were applied to collect the

xylem sap for pH measurements. First, sap was expressed with a pressure chamber (PMS Instruments, Corvallis, OR, USA) from excised twigs. Second, sap was expressed with a vise from 5 mm diameter stem increment cores. The pH of the expressed sap was measured with a solid state pH microsensor connected to a pH meter (Red-Line Standard sensor, Argus meter, Sentron, Roden, The Netherlands). Because sampling was destructive, pH measurements were performed after the experiments and we assumed that the pH was constant during the experiments.

Sap velocity (cm h⁻¹) was measured at two xylem depths (15 and 25 mm) with thermal dissipation probes (Model TDP-30 and TDP-50, Dynamax Inc., Houston, TX, USA) (Granier 1985, 1987) installed on opposite sides of the stem 0.3 m above ground (Figure 7.1). The sensors were thermally insulated with open cell foam and several layers of aluminium foil. Average sap velocity was converted to total sap flow (f_s) (g h⁻¹) by multiplying by the sapwood area.

All sensors and the cuvette were protected from solar radiation by wrapping the stem with reflective bubble insulation (Reflectix; Reflectix Inc., Markelville, IN, USA). Sensor data were collected simultaneously every 10 s and recorded every 300 s using a datalogger (Model CR23X, Campbell Scientific Inc., Logan, UT, USA). In addition, a weather station measured ambient air temperature and relative humidity (CS500, Campbell Scientific Inc., Logan, UT, USA) and rainfall (TE525, Campbell Scientific Inc., Logan, UT, USA) every 300 s and recorded 900-s means (CR10X, Campbell Scientific Inc., Logan, UT, USA). Vapour pressure deficit (VPD) (kPa) was calculated from air temperature and relative humidity data.

7.2.3 Calculation of CO₂ flux components and stem respiration rates

The mass balance approach developed by McGuire and Teskey (2004) was applied to estimate the respiration rate (R_s) ($\mu\text{mol CO}_2 \text{ m}^{-3} \text{ s}^{-1}$) of the stem segment enclosed by the cuvette. This balance consists of three terms: the radial efflux of CO₂ from the stem to the atmosphere (i.e., E_{CO_2}), the axial flux of dissolved CO₂ entering and leaving the segment in the flowing sap (i.e., transport flux, F_T) and the increase or decrease in sap [CO₂*] of the segment over time (i.e., storage flux, ΔS).

$$R_s = E_{\text{CO}_2} + F_T + \Delta S \quad (7.2)$$

Transport flux ($\mu\text{mol CO}_2 \text{ m}^{-3} \text{ s}^{-1}$) was calculated as:

$$F_T = (f_s/V) \times \Delta[\text{CO}_2^*] \quad (7.3)$$

where f_s is the sap flow rate through the segment (l s^{-1}), V is the sapwood volume of the stem segment (m^3) and $\Delta[\text{CO}_2^*]$ is xylem $[\text{CO}_2^*]$ measured above and below the cuvette minus $[\text{CO}_2^*]$ below the cuvette ($\mu\text{mol l}^{-1}$).

Storage flux ($\mu\text{mol CO}_2 \text{ m}^{-3} \text{ s}^{-1}$) was calculated as:

$$\Delta S = ([\overline{\text{CO}_2^*}]_{t1} - [\overline{\text{CO}_2^*}]_{t0}) \times w/\Delta t \quad (7.4)$$

where $[\overline{\text{CO}_2^*}]_{t0}$ is the mean of $[\text{CO}_2^*]$ above and below the cuvette at time $t0$ and $[\overline{\text{CO}_2^*}]_{t1}$ is the mean of $[\text{CO}_2^*]$ above and below the cuvette at time $t1$, w is the specific water content of the segment (l m^{-3}) and Δt is the time interval ($t1 - t0$) (300 seconds). We assumed a sapwood water content of 500 l m^{-3} , based on the average specific gravity for hardwoods (Panshin and de Zeeuw 1980).

7.2.4 Temperature response of stem CO_2 efflux rate and respiration rate

To investigate the temperature response of E_{CO_2} , the data for days without rainfall were used from each tree. E_{CO_2} was regressed against T_{st} ($^{\circ}\text{C}$) with the following equation:

$$E_{\text{CO}_2} = E_{\text{CO}_2}(20) \times Q_{10}^{\frac{T_{\text{st}}-20}{10}} \quad (7.5)$$

where $E_{\text{CO}_2}(20)$ is the CO_2 efflux rate at 20°C and Q_{10} is the relative increase in CO_2 efflux rate with a 10°C rise in temperature. Parameters $E_{\text{CO}_2}(20)$ and Q_{10} were estimated by ordinary least squares. A second regression was performed, with the dataset further limited to periods where f_s was zero (nighttime data).

The same procedure was used to investigate the temperature response of R_s of each tree, using the datasets without rainfall:

$$R_s = R_s(20) \times Q_{10}^{\frac{T_{\text{st}}-20}{10}} \quad (7.6)$$

where $R_s(20)$ is the respiration rate at 20°C and Q_{10} is the relative increase in respiration rate with a 10°C rise in temperature.

Values of T_{st} and the parameters obtained from the regression between R_s and T_{st} were used to predict the respiration rate of the stem segment with Equation 7.6. Residual E_{CO_2} and R_s were calculated as the difference between measured E_{CO_2} or calculated R_s and the temperature prediction based on Equation 7.6.

7.3 Results

7.3.1 Microclimatological and physiological variables

Most days during the experiment were sunny, except for day 290, day 191 (Tree 1) and day 300 (Tree 3), when it rained, and day 289 (Tree 1) and day 292 (Tree 2), when it was heavily clouded (Figure 7.2). Stem temperatures ranged from 6 - 25 °C over the measurement period.

Maximum f_s was 2537, 971 and 910 g h⁻¹ for Tree 1, 2 and 3, respectively (Figure 7.2). It is noteworthy that during some nights, f_s never reached zero. When nighttime VPD remained high (> 0.2 kPa) on days 288 - 289, 294 - 295, 295 - 296, 300 - 301 and 301 - 302, substantial nighttime f_s occurred.

The two different methods for measuring sap pH gave similar mean results: 6.80 ± 0.55 with the pressure bomb and 6.83 ± 0.17 with the vise. Sap pH did not significantly differ between the three trees, so an average value (6.80) was used to calculate xylem [CO₂*]. Xylem [CO₂*] ranged from 2.78 to 9.45, 3.16 to 7.85 and 2.51 to 8.49 mmol l⁻¹ for Tree 1, 2 and 3, respectively (Figure 7.2). This corresponds with a [CO₂] range of 3.9 to 17.6%. Xylem [CO₂*] above the cuvette was generally slightly higher than [CO₂*] below the cuvette. The mean difference was 0.32, 0.31 and 0.32 mmol l⁻¹ for Tree 1, 2 and 3, respectively. Diurnal patterns of both upper and lower [CO₂*] measurements were very similar. On sunny days, xylem [CO₂*] exhibited clear daytime depressions, despite the higher T_{st} during the day than during the night. During rain (days 290 and 300), [CO₂*] increased dramatically. In Tree 3 [CO₂*] more than doubled during the rain, and from the moment f_s restarted (nighttime f_s due to a sudden increase in VPD when rainfall stopped), [CO₂*] showed a large decline, reaching its original value in the late afternoon the next day.

E_{CO_2} ranged from 154 to 673, 146 to 499 and 183 to 475 μmol m⁻³ s⁻¹ for Tree 1, 2 and 3, respectively (Figure 7.2). Expressed per unit stem surface area, these values correspond with 3.4 - 15.0, 2.8 - 9.6 and 3.5 - 9.2 μmol m⁻² s⁻¹, respectively. Unlike xylem [CO₂*], stem E_{CO_2} exhibited temperature-dependent variations on sunny days. Similar to [CO₂*], E_{CO_2} showed a large increase on the rainy days.

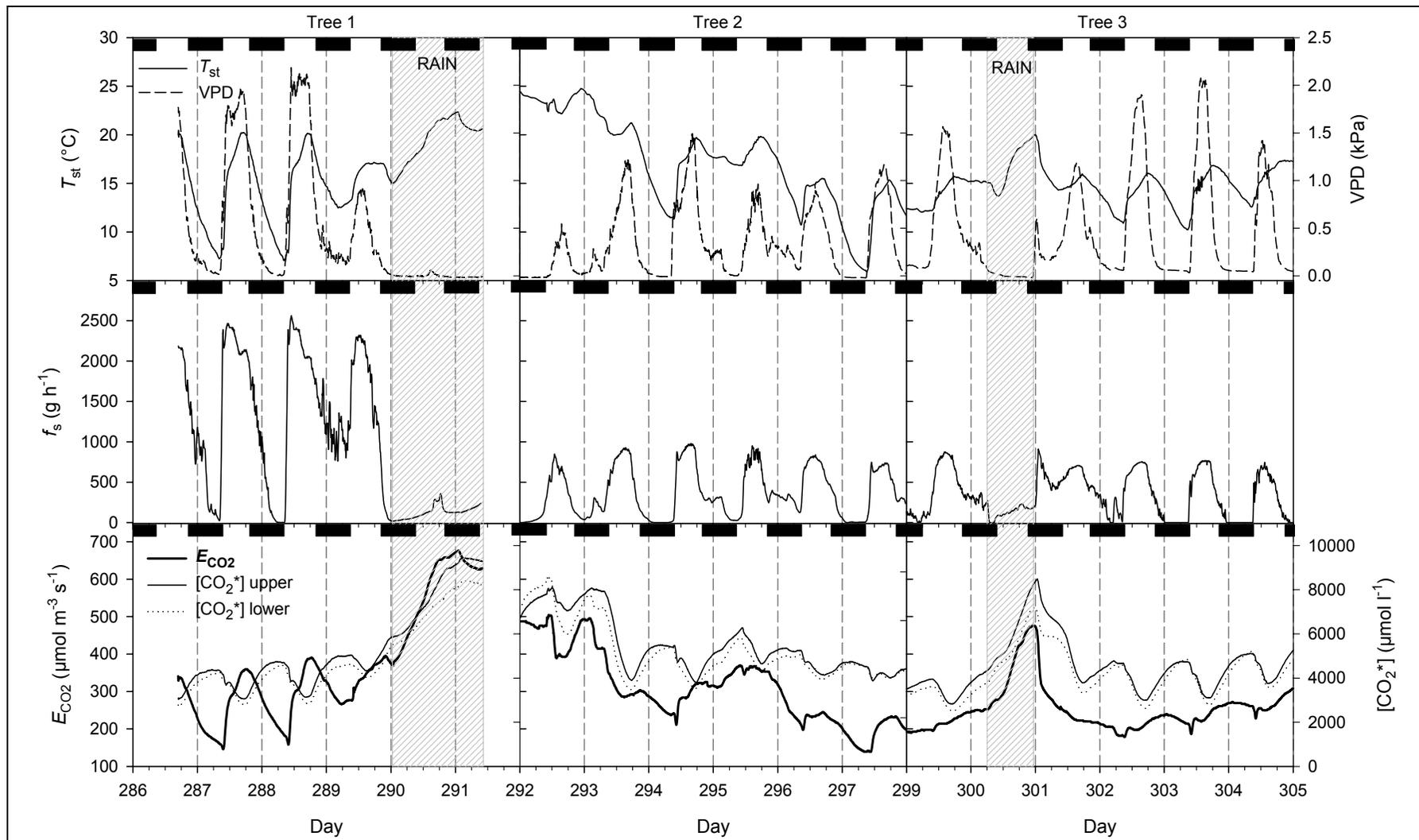


Figure 7.2: Six-day periods of stem temperature (T_{st}), vapour pressure deficit (VPD), sap flow rate (f_s), stem CO_2 efflux rate (E_{CO_2}) and xylem sap CO_2 concentration ($[CO_2^*]$) above and below the stem cuvette as measured in the three three-year-old field-grown trees of a *Populus* clone.

7.3.2 CO₂ efflux rate in relation to temperature

For the entire dataset (except for days with rain), Q_{10} of E_{CO_2} ranged between 1.59 and 1.89, $E_{CO_2}(20)$ between 299.8 and 374.7 $\mu\text{mol m}^{-3} \text{s}^{-1}$ and the coefficient of determination (R^2) for the regression ranged between 0.42 and 0.88 (Table 7.1).

Table 7.1: Characteristics of the response of measured CO₂ efflux (E_{CO_2}) and calculated respiration rate (R_S) to stem temperature (T_{st}) during the entire measurement period, except for days with rainfall, in three trees of a *Populus* clone. R_S was calculated with Equation 7.2 (see text). Q_{10} is the increase in E_{CO_2} or R_S for a 10 °C rise in temperature, $E_{CO_2}(20)$ and $R_S(20)$ ($\mu\text{mol m}^{-3} \text{s}^{-1}$) are the CO₂ efflux rate at 20 °C and respiration rate at 20 °C, respectively (Equations 7.5 and 7.6, see text). Standard errors are given in parentheses. R^2 is the coefficient of determination for the exponential regression between E_{CO_2} or R_S and T_{st} .

	Tree 1			Tree 2			Tree 3		
	Q_{10}	$E_{CO_2}(20)$ or $R_S(20)$	R^2	Q_{10}	$E_{CO_2}(20)$ or $R_S(20)$	R^2	Q_{10}	$E_{CO_2}(20)$ or $R_S(20)$	R^2
E_{CO_2}	1.89 (0.012)	357.5 (1.94)	0.88	1.73 (0.016)	374.7 (1.95)	0.83	1.59 (0.023)	299.8 (2.43)	0.42
E_{CO_2} at $f_s = 0$	2.16 (0.025)	500.8 (6.73)	0.99	1.82 (0.011)	384.1 (1.22)	0.96	1.84 (0.035)	350.9 (4.36)	0.67
R_S	1.90 (0.035)	497.5 (4.87)	0.64	1.91 (0.015)	394.40 (0.96)	0.89	2.20 (0.039)	399.2 (3.88)	0.59

When only data at zero f_s were taken into account, Equation 7.5 was a better fit (higher R^2). Parameter values obtained from the regression based on zero f_s data were higher for all trees (Table 7.1). In Tree 1, measured E_{CO_2} was much lower than the temperature prediction (with parameters obtained for R_S from Table 7.1) during the daytime on sunny days 287 and 288 (Figure 7.3). On average, on these days, measured E_{CO_2} was only 71% of the temperature-predicted flux during daytime (0900 h – 1900 h). Residual E_{CO_2} (i.e., measured E_{CO_2} minus temperature-predicted flux) exhibited a clear diurnal variation, with the lowest values occurring during daytime (Figure 7.4). During the rain (day 290), residual E_{CO_2} was positive, so E_{CO_2} was larger than expected based on temperature. In Tree 2, residual E_{CO_2} exhibited a consistent diurnal pattern on all days: it fluctuated closely around zero during the night and was negative during the daytime. In Tree 3, residual E_{CO_2} also exhibited a clear diurnal pattern, fluctuating around zero or slightly positive during the night and negative during the daytime. Residual E_{CO_2} deviated from this pattern only during the rain (day 300), and was highly positive, indicating that measured E_{CO_2} was larger than what would be expected based on temperature.

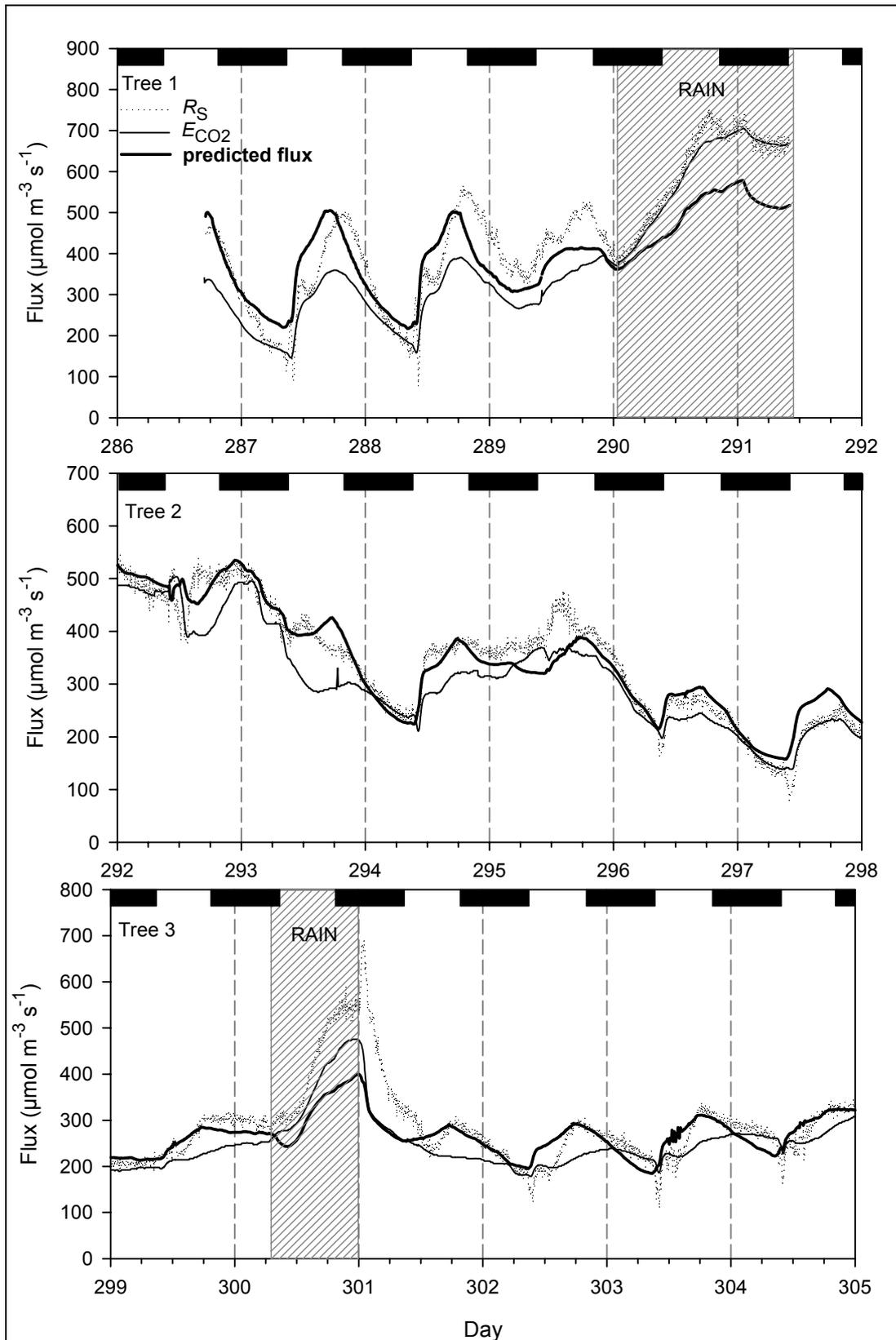


Figure 7.3: Measured stem CO_2 efflux rate (E_{CO_2}), calculated stem respiration rate (R_S) and predicted respiration rate for the three three-year-old field-grown trees of a *Populus* clone. R_S was calculated with Equation 7.2 (see text). The respiration rate was predicted with Equation 7.6 (see text), using Q_{10} and $R_S(20)$ from the temperature response curve of R_S (Table 7.1). Black boxes indicate night periods and vertical dashed lines refer to midnights.

7.3.3 CO₂ efflux rate in relation to xylem CO₂ concentration

The correlation between E_{CO_2} and xylem $[\text{CO}_2^*]$ was unclear. In Tree 1 on sunny days, E_{CO_2} and xylem $[\text{CO}_2^*]$ appeared to be in opposite phase (Figure 7.2). In Tree 2, there was no apparent correspondence between diurnal patterns of E_{CO_2} and xylem $[\text{CO}_2^*]$. In Tree 3, it also appears that $[\text{CO}_2^*]$ and E_{CO_2} were decoupled. E_{CO_2} and xylem $[\text{CO}_2^*]$ were positively correlated only during rainy days (day 290 and 300). In contrast, residual E_{CO_2} correlated fairly well with $[\text{CO}_2^*]$ (Figure 7.4). On the sunny days, the daytime depressions in residual E_{CO_2} corresponded with the reductions in xylem $[\text{CO}_2^*]$ and on the rainy days (day 290 and 300), the large increase in $[\text{CO}_2^*]$ coincided with an increase in residual E_{CO_2} . R^2 values of the linear regression between $[\text{CO}_2^*]$ and E_{CO_2} ranged between 0.63 and 0.94 (Table 7.2).

7.3.4 Calculation of CO₂ flux components and stem respiration rates

Table 7.3 summarizes 6-h mean fluxes of CO₂ and their relative contribution to total R_S for a 24-h period on selected sunny days and rainy days, together with their 24-h totals. Over a 24-h period, E_{CO_2} was by far the most important component on all days, and represented between 82 and 94% of the total flux of respired CO₂, resulting in a generally close correspondence between R_S and E_{CO_2} (Figure 7.3). ΔS and F_T were rather small, and their relative contribution to R_S differed between sunny and rainy days. On the sunny days, ΔS was zero over a 24-h period, indicating that there was no net change in $[\text{CO}_2^*]$ over a 24-h period. On these days, ΔS was positive during the evening and night, whereas it was negative in the morning and afternoon. Hence, CO₂ was stored in the sap during evening and night, and released during the daytime. On the rainy days, ΔS was always positive, indicating that CO₂ was being stored the entire day.

The 24-h contribution of F_T to R_S accounted for 9 - 18% and 3 - 7% of R_S on the sunny and rainy days, respectively. The relative contribution of F_T to R_S was higher on the sunny days because of the higher f_s on those days. On the sunny days, the relative contribution of F_T showed a diurnal variation, whereas during the rain, F_T showed no pronounced diurnal trend. Summarized, during rain the discrepancy between E_{CO_2} and R_S was mainly due to the storage of CO₂ inside the stem, whereas during the daytime of sunny days, the discrepancy was mainly attributed to the transport of CO₂ in the stem.

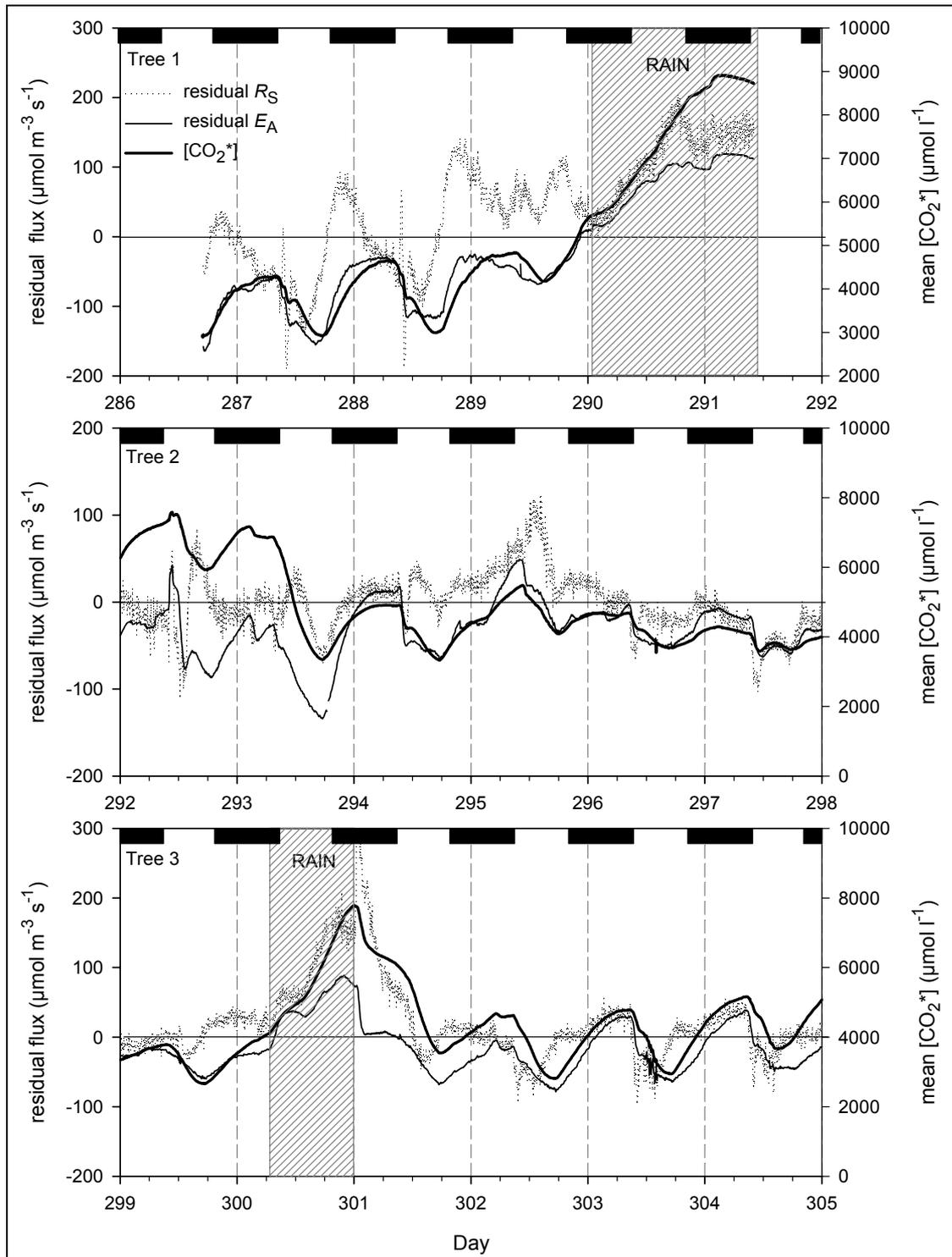


Figure 7.4: Residual stem CO_2 efflux rate (E_{CO_2}), residual respiration rate (R_S) and mean xylem sap CO_2 concentration ($[\text{CO}_2^*]$) for the three three-year-old field-grown trees of a *Populus* clone. Residuals were calculated as the difference between measured E_A or calculated R_S and predicted flux, where the flux was predicted with Equation 7.6 (see text) using Q_{10} and $R_S(20)$ parameters of the temperature response curve of R_S (Table 7.1). Black boxes indicate night periods and vertical dashed lines refer to midnights.

Table 7.2: Coefficients and R² for the linear regression: residual $E_{CO_2} = a \times [CO_2^*] - b$ or residual $R_S = a \times [CO_2^*] - b$. E_{CO_2} (stem CO₂ efflux, $\mu\text{mol m}^{-3} \text{s}^{-1}$) and $[CO_2^*]$ (xylem sap CO₂ concentration, $\mu\text{mol l}^{-1}$) were measured in three trees of a *Populus* clone. R_S (respiration rate, $\mu\text{mol m}^{-3} \text{s}^{-1}$) was calculated with Equation 7.2 (see text). Residuals were calculated as the difference between measured E_{CO_2} or calculated R_S and predicted flux, where the flux was predicted with Equation 7.6 (see text) using Q_{10} and $R_S(20)$ parameters of the temperature response curve of R_S (Table 7.1). Standard errors are given in parentheses.

	Tree 1			Tree 2			Tree 3		
	<i>a</i>	<i>b</i>	R ²	<i>a</i>	<i>b</i>	R ²	<i>a</i>	<i>b</i>	R ²
residual E_{CO_2}	0.043 (0.0003)	243.49 (1.60)	0.94	0.040 (0.0009)	203.79 (4.12)	0.63	0.027 (0.0004)	135.05 (1.90)	0.71
residual R_S	0.032 (0.0008)	124.84 (4.39)	0.54	0.024 (0.0015)	104.55 (6.69)	0.13	0.041 (0.0008)	156.48 (3.51)	0.61

7.3.5 Respiration rate in relation to temperature and xylem CO₂ concentration

Estimated parameter values of $R_S(20)$ and Q_{10} for the fit of Equation 7.6 to calculated R_S are listed in Table 7.1. Generally, the parameters were higher than for E_{CO_2} obtained with the first regression (all data except rain days). R² values were higher than for E_{CO_2} in Tree 2 and 3 but lower in Tree 1. Compared with the second regression of E_{CO_2} (only zero f_s data), parameters were higher for Tree 1, but lower for Tree 2 and 3, and R² values were lower for all trees. Although the temperature prediction (with parameters obtained for R_S from Table 7.1) correlated generally well with R_S , residual R_S deviated several times from zero (Figure 7.4). In Tree 1, on sunny days 287 and 288, residual R_S was negative in the afternoon, indicating that R_S was lower than what would be expected based on temperature. During the rain (day 290), residual R_S was positive and reached 205 $\mu\text{mol m}^{-3} \text{s}^{-1}$. On the other days, the temperature prediction fitted R_S better. In Tree 2, residual R_S was small, and showed no consistent diurnal pattern. In Tree 3, residual R_S showed a diurnal pattern on sunny days 302 - 304: it was zero during the night and negative during the daytime. During the rain (day 300), residual R_S was positive and reached 300 $\mu\text{mol m}^{-3} \text{s}^{-1}$. A positive correlation between residual R_S and xylem $[CO_2^*]$ was found, but the relationship was weaker than for residual E_{CO_2} (Figure 7.4, Table 7.2).

Table 3. Six-h mean (minimum-maximum) flux components (E_{CO_2} = efflux to the atmosphere, F_T = transport flux and ΔS = storage flux) and total respiration rate (R_S), calculated from 5-min measurements; and relative contribution of the flux components to R_S . 24-h totals, calculated from 5-min measurements are also given.

Tree	Time (h)	Flux components and total respiration rate ($\mu\text{mol m}^{-3} \text{s}^{-1}$)				Relative contributions to R_S		
		E_{CO_2}	F_T	ΔS	R_S	E_{CO_2}	F_T	ΔS
Tree 1 day 287 (sunny)	00 - 06	192.1 (171.5-225.5)	30.5 (2.9-67.6)	6.8 (-20.4-34.5)	229.4 (158.4-309.0)	0.84	0.13	0.03
	06 - 12	188.7 (146.0-281.4)	24.3 (0.4-98.3)	-12.4 (-89.8-42.1)	200.6 (89.5-339.7)	0.94	0.12	-0.06
	12 - 18	324.6 (283.1-359.6)	68.6 (43.3-104.3)	-18.0 (-46.5-15.6)	375.2 (292.0-479.1)	0.87	0.18	-0.05
	18 - 00	328.9 (281.4-359.6)	98.3 (64.8-118.7)	27.9 (-5.6-52.4)	455.2 (359.2-505.7)	0.72	0.22	0.06
	24 h total¹	22.4	4.8	0.1	27.3	0.82	0.18	0.00
Tree 1 day 290 (rain all day)	00 - 06	401.1 (368.9-445.8)	1.9 (1.2-2.9)	9.2 (-16-37.2)	412.2 (360.7-480.4)	0.97	0.00	0.02
	06 - 12	493.7 (446.2-540.9)	5.2 (2.8-7.6)	21.4 (-2.9-45.8)	520.3 (460.5-583.8)	0.95	0.01	0.04
	12 - 18	606.5 (542.1-652.9)	22.6 (8.3-51.2)	23.1 (-7.2-64.1)	652.2 (554.2-740.4)	0.93	0.03	0.04
	18 - 00	659.9 (651.5-671.5)	32.4 (22.6-68.0)	15.0 (-21.9-48.3)	707.3 (667.7-753.4)	0.93	0.05	0.02
	24 h total¹	46.7	1.3	1.5	49.5	0.94	0.03	0.03
Tree 2 day 296 (sunny)	00 - 06	271.0 (233.7-317.5)	23.8 (15.7-31.0)	-0.6 (-18.5-22.6)	294.3 (234.7-357.4)	0.92	0.08	0.00
	06 - 12	223.1 (197.3-238.4)	21.0 (5.2-53.8)	-11.5 (-57.8-18)	232.6 (164.0-281.2)	0.96	0.09	-0.05
	12 - 18	237.7 (230.9-244.8)	38.6 (18.0-54.4)	-8.8 (-54.4-18.6)	267.4 (230.1-285.7)	0.89	0.14	-0.03
	18 - 00	219.4 (200.2-235.9)	8.8 (1.5-18.1)	10.7 (-11.5-34.2)	239.0 (190.3-267.7)	0.92	0.04	0.04
	24 h total¹	20.5	2.0	-0.2	22.0	0.93	0.09	0.00
Tree 3 day 299 (sunny)	00 - 06	193.3 (189.6-197.9)	5.2 (0-13.4)	7.6 (-11.4-24.7)	206.1 (186.4-224.3)	0.94	0.03	0.04
	06 - 12	200.6 (194.9-214.2)	23.5 (4.5-51.3)	-4.2 (-51.9-26.4)	219.9 (187.0-255.9)	0.91	0.11	-0.02
	12 - 18	217.6 (214.1-228.0)	54.7 (46.5-86.2)	-15.6 (-41.9-17.7)	256.7 (222.6-304.9)	0.85	0.21	-0.06
	18 - 00	238.3 (227.9-246.8)	46.2 (35.7-62.4)	19.1 (-0.8-32.4)	303.6 (282.3-316.6)	0.78	0.15	0.06
	24 h total¹	18.4	2.8	0.1	21.3	0.86	0.13	0.00
Tree 3 day 300 (rain 06h20 – 22h50)	00 - 06	249.7 (247.2-252.9)	36.3 (20.0-49.7)	12.3 (-11.5-28.9)	298.3 (273.1-315.0)	0.84	0.12	0.04
	06 - 12	273.9 (252.8-294.3)	6.4 (0.0-37.3)	20.2 (-0.1-45.2)	300.5 (270.7-328.6)	0.91	0.02	0.07
	12 - 18	360.1 (295.5-423.6)	22 (8.8-43.3)	30.4 (1-61.3)	412.6 (310.5-522.5)	0.87	0.05	0.07
	18 - 00	457.6 (423.6-475.4)	45.3 (38.5-73.1)	35.1 (-13.1-82.8)	537.9 (503.0-591.1)	0.92	0.04	0.04
	24 h total¹	29.0	2.4	2.3	33.5	0.87	0.07	0.07

¹ Unit for 24-h totals is mol m^{-3}

7.4 Discussion

7.4.1 Xylem CO₂ concentrations

Consistent with previous reports, xylem [CO₂*] was several orders of magnitude higher than the [CO₂] in air (Levy and Jarvis 1998, Teskey and McGuire 2002), indicating that there are large barriers to radial CO₂ diffusion. In most species, the cambium significantly inhibits radial gas diffusion (Kramer and Kozlowski 1979). The xylem itself also forms a barrier to free gas exchange. Sorz and Hietz (2006) found that diffusion coefficients for O₂ in water-saturated xylem were always lower than the diffusion coefficients in water alone, illustrating that the xylem cell walls present a major barrier to gas diffusion. Moreover, it has been demonstrated that differences in [CO₂] between xylem and bark tissues are large: values for xylem [CO₂] have been reported to be as high as 26% (MacDougal and Working 1933), while [CO₂] in bark tissues are reported to be around 0.06 to 0.17% (Cernusak and Marshall 2000, Wittmann et al. 2006). Due to these barriers, respired CO₂ builds up in the xylem instead of directly escaping to the atmosphere. This build-up of CO₂ was clearly visible during the rain events, when [CO₂*] more than doubled (Figure 7.2, day 290 and 300).

A diurnal pattern was observed in xylem [CO₂*] on sunny days. Despite higher T_{st} during the day, [CO₂*] was lower during the day than during the night (Figure 7.2). When f_s was high, the sap in the stem at high [CO₂*] appears to have been diluted with water coming from the soil at lower [CO₂*]. During sunny days, [CO₂*] always decreased as f_s increased (Figure 7.2), suggesting that although the respiration rate of the living xylem cells might be greater during the day due to higher T_{st} , the decrease of xylem [CO₂*] due to dilution by soil water can overwhelm any increase in xylem [CO₂*] due to CO₂ production. However, because we did not measure xylem water status, we cannot exclude a possible decrease in the actual respiration rate of the living stem tissues due to water stress. When transpiration starts, tension develops in the xylem and turgor pressure of the living cells in the stem decreases. A drop in turgor pressure may reduce rates of growth and maintenance processes and the respiratory processes which support those (Woodruff et al. 2004, Proseus and Boyer 2006, Steppe et al. 2006). Water deficits have been used to explain midday depressions in stem CO₂ efflux (Edwards and McLaughlin 1978, Lavigne 1987, Kakubari 1988, Wang et al. 2003, Daudet et al. 2005, Saveyn et al. 2007a). However, it is extremely difficult to distinguish between the effects of CO₂ transport in the xylem and those of turgor dynamics in the living stem tissues because both processes are determined by sap flow (Saveyn et al. 2006). Hence, it is possible that water deficit in the living stem tissues may play a role in the diurnal pattern of [CO₂*].

During rainy days (day 290 and 300), xylem $[CO_2^*]$ increased sharply (Fig. 2). Due to the lack of f_s during the rain, xylem $[CO_2^*]$ was not diluted and transport of CO_2 did not occur, resulting in a build-up of xylem $[CO_2^*]$. However, the large increase in $[CO_2^*]$ in the absence of f_s might also be partly due to a better stem water status and, hence, higher rates of cell metabolism and respiration

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7.4.2 CO_2 efflux rate in relation to temperature

We found that $E_{CO_2(20)}$ and Q_{10} values were lower when calculated with the entire dataset (day and night) compared to calculations with night-only data when f_s was zero (Table 7.1). Furthermore, during the daytime on sunny days, E_{CO_2} was generally lower than expected based on the exponential regression of R_s and temperature, using parameters from Table 7.1 (Figure 7.4). Similar discrepancies have been observed previously in other species, and several explanations for this phenomenon have been proposed: (1) Measurements of T_{st} at one location might not be representative of the temperature of the whole stem, so that temperature predictions of E_{CO_2} are not representative for the whole stem (Stockfors 2000). (2) Bark photosynthesis during daytime may cause re-fixation of CO_2 respired by the living stem tissues (Sprugel and Benecke 1991, Saveyn et al. 2006). (3) Actual respiration rates might be higher at night than during the day because a better stem water status in the absence of f_s at night promotes higher turgor pressure in the living stem tissues and, hence, higher growth and maintenance metabolism (Edwards and McLaughlin 1978, Lavigne 1987, Kakubari 1988, Wang et al. 2003, Daudet et al. 2005, Saveyn et al. 2007a). (4) High f_s during the daytime causes a dilution of xylem $[CO_2^*]$ with water at lower $[CO_2^*]$ coming from the soil, resulting in a decrease in stem xylem $[CO_2^*]$ and, consequently, a decrease in E_{CO_2} due to a reduction in the CO_2 concentration gradient from stem to atmosphere (Boysen-Jensen 1933, Negisi 1979, Hari et al. 1991, Martin et al. 1994, Kaipainen et al. 1998, McGuire and Teskey 2007). Two of these explanations do not apply in the context of this study. Explanation (1) does not apply because the stem was covered with reflective bubble insulation, which blocked direct sunlight and prevented the occurrence of large temperature gradients. We measured T_{st} at two places in the stem and found very similar diurnal patterns. Bark photosynthesis (explanation 2) was also prevented by covering the cuvette with aluminium foil and by covering the entire stem with reflective bubble insulation. Hence, only explanations (3) and (4) may account for

why E_{CO_2} was lower than predicted based on temperature during the daytime on sunny days. However, with our data we could not distinguish between the effects of CO₂ transport in the xylem and those of turgor dynamics in the living stem tissues on E_{CO_2} .

7.4.3 CO₂ efflux rate in relation to xylem CO₂ concentration

On sunny days, a (partial) decoupling between E_{CO_2} and xylem [CO₂*] was observed (Figure 7.2). This decoupling was probably due to a combination of large barriers for radial CO₂ diffusion presented by the cambium and xylem and a predominant contribution of cambial and phloem cell respiration to E_{CO_2} . CO₂ produced by xylem cells is faced with several diffusion barriers (xylem itself, cambium and bark), while CO₂ produced by cambium and phloem tissues must overcome only the diffusion resistance of the bark. The phloem itself is aerated by numerous gas spaces, which have continuity with the external environment (Hook et al. 1972). Therefore, measured E_{CO_2} is most likely primarily related to the respiration rate of the phloem and cambium. A decoupling between xylem [CO₂*] and E_{CO_2} has also been reported by Maier and Clinton (2006) and Saveyn et al. (2007b).

Nevertheless, a part of E_{CO_2} was definitely related to xylem [CO₂*]. When the temperature effect was removed from the data, a diurnal variation in residual E_{CO_2} could still be observed on sunny days (Figure 7.4). This variation was well correlated with the diurnal variation in xylem [CO₂*]. (Figure 7.4, Table 7.2). The daytime decrease in xylem [CO₂*] on sunny days caused a decrease in the radial CO₂ diffusion gradient, resulting in a daytime depression in residual E_{CO_2} . On rainy days (day 290 and 300), residual E_{CO_2} was also well correlated with xylem [CO₂*] (Figure 7.4). The build-up of CO₂ on these days caused a large radial CO₂ diffusion gradient, resulting in a large positive residual E_{CO_2} .

In contrast with our findings, other relationships between E_{CO_2} and xylem [CO₂*] have been reported. Maier and Clinton (2006) found that E_{CO_2} was completely decoupled from xylem [CO₂*]. On the other hand, Teskey and McGuire (2005, 2007) and Steppe et al. (2007) found a consistent linear relationship between E_{CO_2} and xylem [CO₂*]. Our results fall between these extremes. The relationship between E_{CO_2} and xylem [CO₂] may vary with season. Maier and Clinton (2006) performed measurements during spring when cambium and phloem likely respire at a very high rate due to growth processes and highly active phloem transport. Our measurements were in autumn, when the growth rate of the trees was much slower. It is possible that due to lower cambium and phloem respiration rates in autumn, xylem [CO₂*] has a relatively larger influence on E_{CO_2} than in spring. The degree of decoupling between E_{CO_2} and xylem [CO₂*] may also be related to age. Decoupling may be more pronounced in young trees because of the high

secondary growth activity (i.e., high rate of oxidative respiration of cambium and phloem meristems) and smaller ratio of xylem tissue compared to cambium and phloem tissue. In mature trees, with reduced secondary growth and a higher ratio of xylem tissue, E_{CO_2} may be more strongly related to xylem $[\text{CO}_2^*]$. Further investigations are needed on seasonal and age-related variation in the relationship between xylem $[\text{CO}_2^*]$ and E_{CO_2} .

7.4.4 Calculation of CO_2 flux components and stem respiration rate

We found that the relative contribution of E_{CO_2} to R_S in *Populus deltoides* stems ranged between 82 and 94% over a 24-h period. Likewise, Bowman et al. (2005) found that E_{CO_2} in *Dacrydium cupressinum* Lamb stems accounted for 86 - 91% of R_S over a 24-h period. On sunny days, the discrepancy between E_{CO_2} and R_S during the daytime was mainly caused by transport of CO_2 in the transpiration stream. F_T accounted for up to 18% of R_S over a 24-h period, which is similar to the findings of McGuire and Teskey (2004) that F_T was 15 and 14% of R_S in *Fagus grandifolia* Ehrh. and *Liquidambar styraciflua* L. stems, respectively, and with the findings of Bowman et al. (2005) that F_T accounted for 11% of R_S in *D. cupressinum* stems. There was no net change in xylem $[\text{CO}_2^*]$ on sunny days because the CO_2 that built up in the stem during the night was released from storage during the daytime. On rainy days, ΔS was positive during the entire day, indicating that CO_2 entered into storage. The relative contribution of F_T to R_S was very small during rain periods due to negligible f_s . Hence, during rain, the difference between E_{CO_2} and R_S was mainly attributed to storage of CO_2 in the stem.

7.4.5 Respiration rate in relation to temperature

Because R_S accounts for both internal and external fluxes of CO_2 and theoretically represents actual stem respiration, it was expected that there would be a strong correlation between R_S and T_{st} . However, the relationship was rather weak in two of the three trees (Table 7.1). The lack of a strong relationship may indicate that the rate of stem respiration is dependent not only on temperature, but also on other factors, or alternatively, that the estimate of the respiration rate is incomplete. There are a number of possible factors that may affect respiration. One is stem water status. Saveyn et al. (2007a) demonstrated that temperature-independent diurnal dynamics of stem E_{CO_2} in *Fagus sylvatica* L. and *Quercus robur* L. were fairly well correlated with the turgor pressure in the living cells, suggesting that in addition to effects of temperature, respiration was also affected by stem water status. Another factor may be diurnal changes in carbohydrate availability. Rates of leaf and root respiration have been linked to the availability of current photosynthate (Azcon-Bieto and Osmond 1983, Högberg et al. 2001, Johnsen et al. 2007). A similar dependence may exist for stem tissues. Temporal and spatial variation in the concentration of O_2 within the stem (Eklund 2000) may

also affect the rate of respiration (Sorzi and Hietz 2006) and hence, the variability in the relationship of R_S to temperature.

We observed that during the rain, R_S was substantially higher than on sunny days (Table 7.3), and also substantially higher than the temperature prediction as indicated by the positive R_S residuals (Figure 7.4). The most likely explanation for these discrepancies is water stress during sunny days that reduced cell metabolism. On rainy days stem [CO₂*] increased throughout the day, i.e., ΔS was positive, as were E_{CO_2} and F_T . We assumed that E_{CO_2} was measured accurately, and ΔS and F_T were both small relative to E_{CO_2} on rainy days. Therefore it appears that the high R_S on rainy days was an actual biological response and not an error in measurement. On sunny days it is likely that water stress reduced R_S , but it is also possible that the measurements of F_T or ΔS were inaccurate. With the current data, it is not possible to determine if either or both fluxes were underestimated on sunny days. These results suggest the need for manipulative studies under carefully controlled conditions to examine the component fluxes of R_S as well as the effects of stem water stress on R_S .

It should be noted that there are some potential sources of error in our calculations and measurements. One is the assumption of constant xylem sap pH. Diurnal changes in xylem sap pH have been reported (Schurr and Schulze 1995). pH changes have a substantial effect on calculation of xylem [CO₂*] (Henry's law) and, hence, on calculation of F_T and ΔS . Another source of error may originate from drilling the holes for inserting the NDIR probes. The NDIR CO₂ sensor was used because of its good reliability, but it is relatively large compared to a CO₂ microelectrode that can also measure stem [CO₂] but is much less reliable (McGuire and Teskey 2002). We recognize that the 19 mm diameter hole required to insert the probe may have had the potential to cause wound respiration, but we think it had a minimal effect on the measurements because (1) the pattern of response was very similar to previous measurements made with CO₂ microelectrodes inserted in much smaller (4 mm) diameter holes (McGuire and Teskey 2004); (2) we observed the same increase in [CO₂] in the stems of large trees when the probes are inserted on the same or different sides of the tree stem (Teskey and McGuire 2007); and (3) the initial apparent wound response in tree stems appears to be due to an increase in CO₂ diffusion from the stem rather than a substantial change in the actual rate of respiration (Teskey and McGuire 2005). Additionally, the drilled holes may have introduced artificial reductions in f_s and, hence, errors in the calculation of F_T . The overall reduction in sap flow was probably small because it has been demonstrated in *Acer pseudoplatanus* (which like *Populus deltoides* has diffuse-porous xylem anatomy) that substantial reductions in sapwood area (>50%) induced by cutting did not reduce whole plant transpiration (Mackay and Weatherley 1973).

7.5 Conclusions

This study demonstrated that E_A was affected by changes in both T_{st} and xylem $[CO_2^*]$. Changes in xylem $[CO_2^*]$ were not related to changes in T_{st} , but were inversely related to changes in f_s , which probably affected both the rate of CO_2 transport and the water status of the living stem tissues and, hence, the rate of respiration. We conclude that E_A does not equal stem respiration. It is a process affected by respiration, but also by removal of internal CO_2 in flowing xylem sap. E_A was the dominant component flux of R_S calculated by mass balance. However, the internal transport flux also accounted for a substantial portion of stem-respired carbon. Unexpectedly, the relationship between R_S and T_{st} was sometimes weak. Other factors including the water status of the living stem tissues or oxygen and carbohydrate availability may have contributed to variability in R_S . However, it is also possible that errors in the measurement or calculation of the component fluxes of R_S contributed to the large difference in R_S between sunny and rainy days.

Chapter 8

General discussion and future perspectives

8.1 General discussion

8.1.1 *The origin and fate of CO₂ inside tree stems*

When reading reports on respiration of woody tissues, one gets the impression that it is a general notion among tree physiologists that measured CO₂ efflux rate (E_{CO_2}) from the woody tissue surface equals the respiration rate (R_S) of the tissue. This concept has persisted since the earliest measurements of woody tissue E_{CO_2} , despite several indications to the contrary. Already in the 1930's a number of scientists have argued about the validity of this assumption, but because of the complexity of the subject, their assertions have usually been ignored. Chapter 1 of this thesis attempted to merge the previously formulated concerns about interpreting E_{CO_2} measurements as an approximation for stem R_S . Several researchers have hypothesized that not all CO₂ respired by the living stem tissues escapes to the atmosphere. Respiratory CO₂ may be re-fixed by chloroplast containing tissues in the corticular layers and wood. Another option is that due to the high CO₂ partial pressure in the stem, a part of the respiratory CO₂ dissolves in the xylem sap and is exported by the transpiration stream. Furthermore, a small number of researchers have suggested that CO₂ escaping from the stem surface may not solely originate from local respiring cells, but also from soil water and respiring cells in the roots, which is imported in dissolved form by the transpiration stream.

The importance of every source and sink of stem internal CO₂ depends each on different factors. Chapter 1 gave for each source and sink a brief overview of the factors that are known or assumed to affect the contribution of that sink or source to the carbon balance of the tree stem. The major driving variables of stem R_S are growth rate and temperature, but also nutrient and water status and O₂ and carbohydrate availability have been put forward as potential controlling factors. The import and export of dissolved CO₂ with the transpiration stream depend on the flow rate of xylem sap and on the solubility of CO₂ in the sap, which is determined by temperature, pH and the CO₂ partial pressure in the gas phase of the xylem. CO₂ re-fixation relies on the availability of an effective chloroplast

structure, photosynthesizing enzymes, nutrients, water, photosynthetically active radiation and CO_2 . Diffusion of CO_2 to the atmosphere depends on temperature, on the CO_2 concentration gradient between the stem interior and the atmosphere, on the magnitude of the diffusion resistances of xylem, cambium and bark layers and on the ratio gas/water content in the stem. The interplay of all these variables influences the relationship between E_{CO_2} and stem R_S . For example, when temperature rises, one can assume that the increased E_{CO_2} is a reflection of the increased R_S . However, increased temperature also affects other processes such as the rate of CO_2 diffusion and the solubility of CO_2 in the xylem sap. The question arises how we can quantify stem R_S in a proper way. Recently, a mass balance approach has been developed by McGuire and Teskey (2004) to calculate R_S of a stem segment. The basic idea behind the method is that the need for analysis of both external (i.e., E_{CO_2}) and internal fluxes of CO_2 is imperative in the context of quantifying stem respiration. Internal fluxes comprise the transport of dissolved CO_2 with the flowing sap and the change in internal storage of CO_2 .

8.1.2 The achievement of appropriate measurements for stem respiration research

Quantifying the fluxes of CO_2 in a stem segment is not a straightforward task, as pointed out in Chapter 2. External fluxes are relatively easy to measure by use of cuvettes enclosing the stem segment and an infrared gas analyzer. However, for quantifying the internal CO_2 fluxes in a stem segment, there is no ready-to-use technique available. To calculate internal fluxes, measurements of dissolved CO_2 concentrations ($[\text{CO}_2^*]$) should be combined with measurements of mass flux in the xylem. Techniques for measuring mass flow in the xylem are quite common among tree physiological studies, but unfortunately the one ideal technique does not exist. The most appropriate technique for our purpose is the heat balance sensor because it measures the sap flow rate directly, but this technique has the major disadvantage that it can not be applied on large tree stems. Another option is the thermal dissipation probe, but this sensor measures sap flow only at one point in the sapwood, and it is assumed that sap flow is uniform across the sapwood. This assumption has been found to be invalid, implying that errors may be introduced when this technique is used for estimating sap flow rate. Besides sap flow rate, xylem sap $[\text{CO}_2^*]$ needs to be measured. Recent introduction of new sensors in tree physiological studies has enabled precise and continuous measurements of CO_2 concentrations in the gas phase of the xylem ($[\text{CO}_2]$). Both the CO_2 microelectrodes and the non-dispersive infrared CO_2 sensors allow us to trace daily dynamics in xylem $[\text{CO}_2]$, which could previously not be achieved with the discontinuous sampling methods. However, both techniques encounter shortcomings: CO_2 microelectrodes show drift problems, so that they cannot be used for long time periods; non-dispersive infrared sensors are able to function

for long periods, but the dimensions of the sensor are large, so that they can not be applied in small tree stems. Both sensors measure CO_2 concentrations in the gaseous phase of the xylem. To convert $[\text{CO}_2]$ to $[\text{CO}_2^*]$, temperature and pH of the sap need to be known. We tested two methods for sampling xylem sap (expressing sap from excised twigs with a pressure bomb and from stem cores with a vise) and found similar results. Because of the destructive nature of the measurements, the number of samples taken was limited and it was assumed that pH was constant during the day or measurement period. However, this assumption may be incorrect, so that errors are introduced when calculating $[\text{CO}_2^*]$. Therefore, when calculation of the mass balance was not strictly necessary in the context of the study, we did not convert $[\text{CO}_2]$ to $[\text{CO}_2^*]$.

An important factor affecting stem R_S is the growth rate of the tissue. Linear variable displacement transducers (LVDTs) were used to continuously measure radial stem growth and they proved to be a reliable instrument for this purpose. The stem water status has been put forward as another factor affecting stem R_S . LVDTs provide indirect information on this variable as they measure the reversible shrinkage and swelling pattern of the stem that reflects changing levels in hydration. Another indicator for the stem water status is stem water potential. This variable was measured using the pressure bomb and the thermocouple psychrometer. Although the thermocouple psychrometer is a promising tool because it enables non-destructive continuous water potential measurements, it was found at times to exhibit a delayed response and it failed to function in a temperature changing environment.

8.1.3 Stem CO_2 efflux and xylem CO_2 concentration in the leafless season

Because sap flow has been surmised to affect stem E_{CO_2} , it was a logical first step to perform E_{CO_2} measurements when sap was not flowing, i.e., in the leafless season. Measurements of stem E_{CO_2} (using cuvettes covered with aluminium foil to exclude light) and xylem $[\text{CO}_2]$ were performed on a young oak and beech tree grown under a constant light/dark and temperature regime. Although stem temperature (T_{st}) was constant, E_{CO_2} was significantly higher during the dark compared to the light period. A similar daytime reduction in xylem $[\text{CO}_2]$ was observed. Theories for daytime depressions found in literature failed to explain our results: respiratory CO_2 could not be exported by the transpiration stream since no sap flow occurred at the time of measurement and also stem water deficit was unlikely to cause the daytime reductions in E_{CO_2} and xylem $[\text{CO}_2]$ since no significant difference was observed in stem transpiration rates between the light and dark period, and the increase in E_{CO_2} for a 10 °C rise in temperature (Q_{10}) was not consistently lower during the light period compared to the dark. We suggest that corticular and/or wood photosynthesis in the stem

parts adjacent to the cuvette may have influenced E_{CO_2} of the stem part covered by the cuvette by creating an axial CO_2 gradient between the covered and uncovered part of the stem and thus causing axial diffusion from the covered part towards the uncovered part. It was also demonstrated that drilling a hole in the stem below the cuvette largely affected E_{CO_2} of the stem segment enclosed by the cuvette, suggesting that escape of CO_2 through the wound caused a CO_2 gradient along the stem, which in turn caused axial CO_2 diffusion. In the remainder of this thesis, when E_{CO_2} was measured, not only the cuvettes were covered, but also a large part of the stem was covered in order to eliminate the influence of CO_2 re-fixation on E_{CO_2} measurements, and when holes needed to be drilled into the stem to install sensors, putty adhesive was used to seal the hole in order to avoid CO_2 'leakage' through the wounds.

8.1.4 The relationship between stem CO_2 efflux and turgor

Sap flow has been put forward as a factor affecting E_{CO_2} because respiratory CO_2 can be dissolved in the xylem sap and exported away from the site of respiration. However, another consequence of sap flow is depletion of the stem water reserves. In young tree stems, water reserves are mainly located in the tissues external to the xylem, where a large fraction of the cells are living. Depletion of water reserves in these tissues may result in water deficit during the day, slowing down growth and maintenance processes. Particularly growth processes are known to be very sensitive to drought stress. When growth is constrained, so are the respiratory processes which provide energy to support growth. However, analysis of water status in the living stem tissues is not easily accomplished, precluding quick identification of stem water deficit. A proper indicator of water status in the living stem tissues is turgor because it is a driving variable for cell growth, but good techniques for non-destructive measurements of turgor are lacking. In Chapter 4, stem E_{CO_2} measurements were performed on a young oak and beech tree under controlled environmental conditions. The mechanistic flow and storage model RCGro was applied to simulate changes in turgor in the living stem tissues external to the xylem and to determine during which periods of the day growth was constrained by turgor. Variations in temperature-normalized E_{CO_2} were found to correlate fairly well with variations in turgor. Depressions in E_{CO_2} coincided with periods where turgor was lower than the threshold for irreversible growth to occur. However, stem E_{CO_2} also responded to changes in turgor when turgor was still lower than the threshold, suggesting that not only growth but also maintenance processes were affected by the daily dynamics of the water status in the living stem tissues. With the available data we could not distinguish between the two effects of sap flow on E_{CO_2} . Both effects may equally well explain the daytime depressions in stem E_{CO_2} .

8.1.5 Stem CO₂ efflux and xylem CO₂ concentration under drought stress

In Chapter 5, we artificially decreased the stem water status by subjecting a young oak tree to soil water depletion under controlled environmental conditions. Before the drought, stem E_{CO_2} and xylem $[\text{CO}_2]$ correlated fairly well with T_{st} and no daytime depressions were observed. Hence, in this case sap flow had no visible effect on E_{CO_2} or xylem $[\text{CO}_2]$. Three days after the imposition of drought daily growth became zero, indicating that turgor in the living stem tissues was lower than the threshold for irreversible growth to occur. At that time, diurnal patterns of E_{CO_2} and xylem $[\text{CO}_2]$ were no longer controlled by temperature and clear daytime depressions were observed. The diurnal pattern of E_{CO_2} correlated very well with the diurnal variation in stem diameter. Stem diameter variations reflect the shrinkage and swelling pattern of stem water reserves and are thus an indirect indicator for the water status of the stem. Therefore, it is likely that stem E_{CO_2} was affected by diurnal changes in metabolic activity due to changes in stem water status. Because growth did not occur at that time, it appears that also maintenance processes were sensitive to changes in stem water status. Q_{10} of stem E_{CO_2} was found to be lower during drought than during the pre-drought period, confirming the reduced metabolic activity of the living cells. If export of respiratory CO₂ by flowing sap would have caused the daytime depressions, it follows that reductions would be maximum under highest flow rate. Nevertheless, depressions were not observed before drought, when sap flow rates were greatest. When the tree was re-watered, a sharp increase in stem E_{CO_2} and xylem $[\text{CO}_2]$ was observed, which resulted most probably from the increased metabolic activity in the local living stem tissues, but also import of CO₂ by the transpiration stream originating from increased soil and/or root metabolism could have contributed to the increase in E_{CO_2} and $[\text{CO}_2]$. A notable finding of the study is that xylem $[\text{CO}_2]$ was not always strictly correlated with E_{CO_2} . A combination of large barriers for radial CO₂ diffusion presented by the cambium and xylem and a predominant contribution of processes occurring in the cambial and phloem regions to E_{CO_2} may have caused this decoupling.

8.1.6 Variability in stem CO₂ diffusion resistance

In Chapter 6 it was further explored how xylem $[\text{CO}_2]$ and stem E_{CO_2} are related to each other through artificially introducing CO₂ enriched water in eastern cottonwood tree stems under controlled environmental conditions. In all trees, introduction of CO₂ enriched water at the stem base was effective in changing xylem $[\text{CO}_2]$ at 0.5 m above soil level, demonstrating that CO₂ was transported with the flowing xylem sap. The parallel change in E_{CO_2} and xylem $[\text{CO}_2]$ indicates that changes in xylem $[\text{CO}_2]$ strongly affected stem E_{CO_2} . The degree of response of E_{CO_2} to increased xylem $[\text{CO}_2]$ provides information on the magnitude of

resistance for CO₂ to diffuse from xylem to atmosphere. Although the examined trees were of the same clone, tree-specific resistance to radial CO₂ diffusion varied 6-fold. This indicates that the amount of CO₂ retained in the xylem versus that which diffuses to the atmosphere can considerably differ among trees, even if they are genetically identical. Another consequence is that the total amount of CO₂ that diffuses to the atmosphere will consist of varying portions of CO₂ respired by local cells and CO₂ imported by sap flow. Hence, the resistance to CO₂ diffusion affects to which extent it is mistaken to assume that E_{CO_2} reflects R_S . In trees with large resistances, CO₂ transport will be an important issue, so that the assumption will be less valid compared to trees with small resistances. Large variability in diffusion resistance may be an overlooked cause for the large variability in stem E_{CO_2} observed among trees.

The study also showed the diurnal pattern of xylem [CO₂] and E_{CO_2} in a non-manipulated tree. Diurnal patterns of both variables were very similar and exhibited clear daytime depressions, which correlated very well with stem diameter variations, suggesting that water deficit caused a decline in respiration rate. However, it is also possible that dilution of the xylem sap because of import of soil water at much lower [CO₂] caused the daytime reduction in xylem [CO₂] and stem E_{CO_2} .

8.1.7 Stem respiration and CO₂ efflux in relation to temperature and xylem CO₂ concentration

In Chapter 7 we further aimed at deepening our understanding on how stem E_{CO_2} and xylem [CO₂] relate to each other in eastern cottonwood trees under different natural weather conditions. Furthermore, R_S was calculated with the mass balance method (McGuire and Teskey 2004) and the relationship between both E_{CO_2} and R_S and T_{st} was investigated. E_{CO_2} correlated well with T_{st} , except on rainy days and during the daytime of sunny days. At first sight, E_{CO_2} appeared to be largely decoupled from [CO₂*]. However, when the temperature effect was removed from the E_{CO_2} data, the relationship between residual E_{CO_2} and [CO₂*] was strongly positive. Changes in [CO₂*] were not related to changes in T_{st} but were inversely related to sap flow, most probably because sap flow affected the transport rate in the xylem of respiratory CO₂. However, it can not be excluded that sap flow also affected the water status and, hence, the respiration rate of the living xylem tissues. Hence, in this case it appears that stem E_{CO_2} was primarily related to the respiration of the external stem tissues, but that E_{CO_2} was also partially affected by the processes occurring in the xylem. Xylem CO₂ transport may explain temporal inconsistencies in stem E_{CO_2} (day versus night, sunny days versus rainy days, etc.), but also unexpected variability in stem E_{CO_2} between trees and across stands. Strongly transpiring trees or stands (due to a larger canopy for example) are able to transport a relatively greater portion of

respiratory CO_2 , so that stem E_{CO_2} will be more affected compared with weakly transpiring trees or stands. E_{CO_2} was found to be the largest component flux of R_s . Because calculated R_s accounts for both external and internal fluxes of CO_2 , it was expected that R_s would correlate better with T_{st} than E_{CO_2} . R_s correlated generally well with T_{st} , but not on all times. Particularly during rain, R_s was much higher than expected from temperature, which may be the result of improved cell metabolism due to a better stem water status. However, it should also be considered that errors in measurements or in the calculation of R_s contributed to the observed discrepancy between R_s and T_{st} .

8.2 Ecophysiological implications

8.2.1 *Xylem CO_2 concentration measurements reveal previously unknown internal dynamics of carbon inside tree stems*

Recent advances in measuring techniques have facilitated precise and continuous measurement of xylem CO_2 concentrations. Using such methods, a growing number of studies, including those presented here, have revealed previously unknown patterns of xylem $[\text{CO}_2]$. Discontinuous sampling techniques already showed that xylem $[\text{CO}_2]$ in tree stems can reach levels up to 10^3 times higher than ambient $[\text{CO}_2]$, strongly suggesting that CO_2 builds up in the xylem instead of directly diffusing to the atmosphere. By use of continuous measuring techniques it has now emerged that the pool of CO_2 in the xylem exhibits daily fluctuations which may depend on several factors, such as corticular photosynthesis, temperature and sap flow. Surveillance of the dynamic pool of CO_2 in the xylem holds much scope for deepening our understanding on the production and fluxes of CO_2 in the xylem.

8.2.2 *Corticular photosynthesis and wounding interfere with CO_2 efflux measurements*

The dormant season study (Chapter 3) revealed that corticular and/or wood photosynthesis in stem parts adjacent to a stem segment enclosed by a cuvette that excluded all light may affect the E_{CO_2} of that stem segment. This may complicate investigations on stem respiration. However, by covering a large part of the stem above and below the studied stem segment, this problem can be circumvented. The extent to which corticular and/or wood photosynthesis may hamper E_{CO_2} measurements depends on the age of the stem. Effectiveness of corticular photosynthesis decreases dramatically with age, mainly due to the development of thicker, light-reducing outer bark layers (Pfanz et al. 2002). Hence, particularly when measuring E_{CO_2} of young greenish stems or branches precaution should be taken.

In this study it was further observed that drilling a hole 1 cm below the stem cuvette, resulted in a sharp decrease in E_{CO_2} of the stem segment. Hence, drilling a hole and removing a barrier for CO_2 diffusion has a substantial effect on E_{CO_2} at places in the neighbourhood of this hole. An increase in E_{CO_2} following wounding has been observed in woody tissues (Oohata et al. 1967, Levy et al 1999) and leaves (Godwin 1935) and has been associated with an increased respiration rate due to the initiation of repair processes by the injured tissue. However, excision or cutting of tree organs introduces pathways for CO_2 diffusion, facilitating escape of CO_2 to the atmosphere. Similar evidence for this has been given by Teskey and McGuire (2005). They found that stem E_{CO_2} of wounded tissue increased 4- to 7-fold. Their results, together with the results presented here, ask for a re-interpretation of the wound respiration phenomenon. Furthermore, interpretation of stem E_{CO_2} measurements as an indicator of stem respiration in stem girdling experiments (i.e., stripping a band of bark) should be treated with caution: changes in E_{CO_2} after girdling may be rather caused by removal of diffusion barriers than by changes in stem respiration.

8.2.3 Stem respiration models need to include stem water status as a controlling factor

In Chapter 4 it was demonstrated that sap flow may affect stem respiration rates by causing depletion of water reserves in the living stem tissues external to the xylem. In the drought stress study (Chapter 5) it was found that diurnal dynamics of stem E_{CO_2} , which correlated very well with T_{st} in the pre-drought period, became strongly correlated with stem diameter variations during drought, indicating that stem respiration was severely affected by stem water status under limiting water availability. It was also found that xylem $[CO_2]$ showed clear daytime depressions under drought, where it did not in the pre-drought period, suggesting that also the living tissues of the xylem were affected by water deficit.

This implies that a simple exponential temperature function is not sufficient to predict stem respiration. A model should be developed to describe stem E_{CO_2} efflux as a function of both stem temperature and the water status of the living stem tissues. However, it remains difficult to quantify the water status of living tissues. Stem diameter and sap flow measurements in combination with the RCGro model are capable of simulating the pattern of turgor in living stem tissues external to the xylem. Simulations of turgor provide key insights into the water status of living stem tissues, and are therefore a powerful tool for advancing our understanding of the metabolic activity of living stem tissues and the dynamics of stem respiration.

8.2.4 *CO₂ efflux measurements alone are not sufficient to quantify stem respiration*

The drought stress study (Chapter 5) revealed that patterns of stem E_{CO_2} and xylem $[\text{CO}_2]$ were sometimes decoupled. A similar (partial) decoupling between stem E_{CO_2} and xylem $[\text{CO}_2]$ was observed in the field study (Chapter 7): dynamics of the xylem CO_2 pool affected E_{CO_2} , but the effect of temperature on E_{CO_2} was much larger. It appears that stem E_{CO_2} rather reflected the processes occurring in the external tissues than the processes going on in the xylem, probably because of a combination of large barriers for radial CO_2 diffusion presented by the cambium and xylem and a major contribution of processes in the cambial and phloem regions to E_{CO_2} . This implies that measurements of stem E_{CO_2} are not representative for the respiration of the whole stem tissue because xylem respiration is not entirely accounted for. In that case, it is strongly recommended that stem E_{CO_2} measurements are combined with xylem $[\text{CO}_2]$ measurements in order to obtain comprehensive knowledge on the respiration of all living stem tissues.

8.2.5 *Transport of CO₂ with the transpiration stream asks for a re-interpretation of CO₂ exchange measurements*

The sap manipulation experiment (Chapter 6) demonstrated that introducing CO_2 enriched water at the stem base could alter xylem $[\text{CO}_2]$ at 0.5 m height, strongly suggesting that xylem CO_2 can be transported with the flowing xylem sap. This has a number of consequences for several ecophysiological processes that are measured by gas exchange. First, when CO_2 originating from root or stem respiration is transported to the leaves, it may function as an internal CO_2 source for leaf photosynthesis. This implies that measurements of CO_2 uptake from the atmosphere by the leaves probably underestimate the actual leaf photosynthesis. A second picture that needs to be redrawn is that of soil respiration. When measuring soil CO_2 efflux, it is assumed that the sources of CO_2 in the soil are respiration of the roots and their symbiotic mycorrhizal fungi and of microbial and faunal populations living in the soil. However, Burgess and Bleby (2006) recently demonstrated that redistribution of soil water by roots is mediated by stem tissues. They found that xylem sap was able to flow from the stem to the roots and into the soil. This implies that, together with the xylem water also dissolved CO_2 originating from stem respiration enters the soil and contributes to soil CO_2 efflux. In this context it should also be noted that during transpiration, together with the soil water, also dissolved soil CO_2 is taken up by trees instead of contributing to soil CO_2 efflux. The underestimation of soil respiration due to this process may be particularly large on sunny days in summer when sap flow is high.

8.2.6 Contribution of stem respiration to forest carbon balance: larger than previously estimated?

The field study (Chapter 7) revealed that on a 24-h basis, E_{CO_2} was always lower than R_S , indicating that E_{CO_2} measurements always underestimated the 'actual' respiration of the stem segment. Attempts to quantify the contribution of woody tissue respiration to the total carbon balance of forest ecosystems are generally based on a number of E_{CO_2} measurements of branch and stem segments (e.g., Lindroth et al. 1998, Granier et al. 2000, Damesin et al. 2002, Hamilton et al. 2002). Our findings indicate that these values probably underestimate the actual contribution of woody tissue respiration and ask for other methods to quantify woody tissue respiration. The method developed by McGuire and Teskey (2004) is a first promising step towards more accurate estimations of stem respiration.

In the majority of the reports on woody tissue E_{CO_2} measurements, E_{CO_2} is used as a synonym for respiration. We hope that our study may convince scientists to adopt their terminology.

8.3 Areas of future research

Research often evokes more questions than it gives answers and this thesis forms definitely no exception. The subject of this thesis is new, which makes the research challenging, but at the same time not straightforward. Several interesting phenomena were brought out, but it has been difficult to isolate general trends or to provide strong evidence for the hypotheses that were put forward. It is therefore not surprising that our list for future research areas is extensive.

A major thread throughout the different experiments conducted has been the interpretation of patterns of xylem $[CO_2]$ and E_{CO_2} and the interaction among them.

An area of controversy that has emerged from our measurements is the cause of changes in the pool of CO_2 in the xylem. In the dormant season study (Chapter 3), xylem $[CO_2]$ followed the light-dark pattern, suggesting that xylem CO_2 was internally re-fixed in corticular and/or wood photosynthesis. In the drought stress study (Chapter 5) xylem $[CO_2]$ in the pre-drought period correlated very well with the daily pattern of temperature, suggesting that changes in the pool of CO_2 were mainly determined by the respiration rate of the living stem cells, which was strongly temperature-driven. During drought, xylem $[CO_2]$ followed the diurnal pattern of stem diameter, also suggesting that the pool of CO_2 was affected by the respiration rate of the tissue, which in this case was mainly determined by the stem water status. In the fourth study (Chapter 6), the diurnal pattern of xylem

[CO₂] in the non-manipulated tree also correlated very well with stem diameter changes, suggesting that the stem water status affected respiration rate and, hence, the CO₂ pool in the xylem. However, in this case it is also feasible that dilution of the xylem sap because of import of soil water at much lower [CO₂] affected xylem [CO₂]. In the field study (Chapter 7), xylem [CO₂*] showed a diurnal pattern in opposite phase with sap flow rate. Similar patterns were observed in the field studies of McGuire and Teskey (2002) and Teskey and McGuire (2002). It was most likely that xylem sap was diluted with soil water at much lower [CO₂*] than the xylem sap. However, it cannot be excluded that the actual respiration rate of the xylem parenchyma decreased due to water deficit because of low moisture content in the xylem. The various patterns observed among the studies and the different feasible interpretations ask for more in-depth studies on the factors affecting the large pool of CO₂ in the xylem. Clarifying ambiguities about the dynamics of this internal carbon pool may yield insights about the internal fluxes of carbon in tree stems.

The patterns of stem E_{CO_2} observed across the different studies were at least as much unpredictable as patterns of xylem [CO₂]. In the dormant season study (Chapter 3) and sap manipulation study (Chapter 6), stem E_{CO_2} was closely related to changes in xylem [CO₂] suggesting that E_{CO_2} is strongly influenced by processes occurring in the xylem. A strong relationship between xylem [CO₂] and E_{CO_2} has also been observed by Teskey and McGuire (2002, 2005). Gansert and Burgdorf (2005) put it strongly by suggesting that stem E_{CO_2} may originate physico-chemically from a strong diffusion gradient between xylem and atmosphere, rather than from actual respiration of living tissues. Contrarily, in the drought stress study (Chapter 5) and the field study (Chapter 7), E_{CO_2} was partially decoupled from xylem [CO₂], suggesting that E_{CO_2} was primarily related to processes occurring in the external living stem tissues (phloem and cambium). Restricted radial gas movement from xylem to atmosphere due to large diffusion barriers and a predominant contribution of processes occurring in cambium and phloem to E_{CO_2} is likely the cause of this partial decoupling. A total decoupling between stem E_{CO_2} and xylem [CO₂] has been observed by Maier and Clinton (2006). They related this to the very high respiration rates of cambium and phloem during spring, which rules out the contribution of processes occurring in the xylem to stem E_{CO_2} . The different results obtained in this and other studies ask for further investigations on the relationship between xylem [CO₂] and E_{CO_2} . Issues such as seasonal differences in the relationship and differences in the relationship within and among trees or among species need to be addressed. The relationship between E_{CO_2} and xylem [CO₂] involves useful information for stem respiration research because it indicates to which extent the assumption is violated that E_{CO_2} equals total stem respiration. Another major challenge will be to isolate the physical factors controlling the rate of radial CO₂ diffusion. Only when

we can predict the influence of these factors on E_{CO_2} and we can remove the effects of these factors from E_{CO_2} data, we will be able to derive information on stem respiration from E_{CO_2} measurements. However, it is extremely complex to examine the influence of different factors on E_{CO_2} *in situ* without affecting the respiration process at the same time.

Besides these major issues encountered across the different studies, each study has also raised particular questions.

In the literature review (Chapter 1) several potential sources and sinks of stem internal CO_2 have been enumerated. However, it is important to note that these are only the sources and sinks that have been distinguished in literature. We must realize that this list is probably incomplete. For example, given the high respiratory potential of external stem tissues, a potential important source of respiratory CO_2 may be the phloem stream. Increased resolution of measurements of $[CO_2]$ in stem tissues, including cambium and phloem, is needed to expand our current knowledge on the sinks of stem CO_2 . Another potential source and/or sink of CO_2 which has not yet been considered may be the axial diffusion of CO_2 . Also other unknown fluxes may contribute to the import and export of stem internal CO_2 . It will be a challenge to further fill in the gaps in our understanding on the different origins and destinies of CO_2 in tree stems.

In the dormant season study (Chapter 3) it was hypothesized that axial diffusion of CO_2 may affect E_{CO_2} of an enclosed stem segment. Our knowledge on rates of axial gas diffusion in wood is limited to the O_2 diffusion experiments of Sorz and Hietz (2006). They found that O_2 diffusion in wood was faster in axial direction than in radial direction, which makes the hypothesis plausible. However, these experiments were performed under a large axial O_2 gradient (20.95%). The axial gradient in the xylem encountered in our study was probably much smaller. It should be investigated how fast CO_2 can axially diffuse under a rather small CO_2 gradient. In our study we hypothesized that the CO_2 gradient originated from cortical photosynthesis in the stem parts adjacent to the enclosed segment. It needs to be further examined whether photosynthesis is actually capable of causing an axial gradient in xylem $[CO_2]$. It would be intriguing to determine whether different light intensities can alter the axial CO_2 gradient and thus change the magnitude of depression in E_{CO_2} .

In Chapter 4 we demonstrated that daytime depressions in E_{CO_2} correlated very well with diurnal changes in turgor of the living tissues external to the xylem, suggesting that reduced stem water status may limit energy requiring processes in living stem tissues, resulting in lower respiration rates. However, the study could not exclude the other main hypothesis about daytime depressions in E_{CO_2} , namely the export of respiratory CO_2 by the flowing xylem sap, because the two

processes are closely knit. It remains an area of controversy and future studies will need to unravel the relative importance of both processes. Another shortcoming of the study is that the RCGro model only simulates changes in turgor of stem tissues external to the xylem. Although the fraction of living cells in the xylem is relatively small (particularly in small trees), these living xylem cells may also be affected by a decreased turgor as a result of sap flow. It remains to be investigated how changes in turgor in the xylem living cells may affect stem respiration and E_{CO_2} measurements.

The sap manipulation study (Chapter 6) revealed that diffusion resistances vary substantially between trees, even if they are genetically identical. It would be interesting to investigate whether the resistance also differs within a tree (e.g., stem versus branches) and whether the resistance changes throughout the year. If the latter is true, this implies that previously observed seasonal trends in woody tissue E_{CO_2} may rather result from changes in diffusion resistance than from actual changes in respiration. Diffusion resistances of stems and branches are likely to differ because of the thicker bark of stems compared to branches. If these differences are substantial, they may explain the large variability in E_{CO_2} between stems and branches as observed in many studies (e.g., Sprugel 1990, Damesin et al. 2002).

Another interesting area for further research is to determine the physical factors in stems that influence the resistance to radial CO_2 diffusion. It is known that increasing the water content of the wood greatly slows down radial diffusion (Sorz and Hietz 2006) because diffusion in water is 10^4 times slower than in air. Hence, the resistance is expected to be determined not only by the arrangement of cell walls, the structure of the cell wall matrix and/or pit membranes, but also by the ratio gas/water content. This ratio is likely to be higher during the day due to depletion of water reserves. We found that the resistance to radial CO_2 diffusion was indeed higher during the night than during the day. We are unaware of any studies on the diurnal dynamics of the ratio gas/water content and on how these dynamics may influence the diffusion rate.

The field study (Chapter 7) revealed that the relationship between calculated R_S (based on the mass balance approach) and T_{st} was not better than between E_{CO_2} and T_{st} . This suggests that besides temperature also other factors control respiration rates. Because of the much higher R_S than expected from temperature on rainy days, it appears that drought stress limited R_S on sunny days, so that water status may be a controlling factor of R_S . Another plausible explanation is that measurements of the transport flux and storage flux and, hence, calculations of R_S were inaccurate. With the current data, it was not possible to determine if either or both fluxes were erroneous. These results

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suggest the need for more in-depth studies on the transport and storage flux components of R_S as well as the effects of stem water status on R_S .

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Summary

Respiration (R_S) has been put forward as one of the key factors determining whether forests will act as a net source or a net sink of carbon (Lindroth et al. 1998, Valentini et al. 2000) and R_S of stems and branches has been estimated to account for up to one third of the total carbon loss by forest ecosystem respiration (Damesin et al. 2002). Despite its importance to forest ecosystem carbon balances, R_S of woody tissues is poorly understood. The main obstacle for addressing the unanswered questions on woody tissue R_S is the technical difficulty to obtain respiration measurements. A predominant assumption advocated in most studies is that R_S of a stem or branch segment can be quantified by measuring the CO_2 efflux (E_{CO_2}) from that segment. In the majority of the studies E_{CO_2} is even used as a synonym for respiration. However, it has been frequently reported that E_{CO_2} from stems and branches exhibits 'unexpected' patterns, such as temperature-independent diurnal variations or extremely large variability within and among trees and across stands. Today scientists still fail to unequivocally predict and scale woody tissue E_{CO_2} . This may be due to an inadequate understanding of the factors controlling woody tissue R_S , but also because E_{CO_2} simply does not reflect the actual R_S .

The first chapter of this thesis fuses the today's available literature on the sources and sinks of stem internal CO_2 . A major source of stem internal CO_2 is R_S of the living stem cells. Furthermore, CO_2 coming from soil water and/or from root respiration may be imported by the transpiration stream and may enrich the CO_2 concentration inside tree stems. A well-known sink of stem internal CO_2 is radial diffusion to the atmosphere (i.e., E_{CO_2}). However, stem internal CO_2 may also be re-fixed by corticular and/or wood photosynthesis or it may be exported by the transpiration stream. The chapter discusses the potential difficulties that have been identified in literature by using stem E_{CO_2} as a measure of stem R_S . A major concern is that transport of CO_2 by the transpiration stream may bias the relationship between E_{CO_2} and R_S . The chapter ends with an explanation of the method that has recently been developed (McGuire and Teskey 2004) to quantify the actual R_S of a stem segment. This method accounts for both external (i.e., E_{CO_2}) and internal fluxes of respiratory CO_2 .

Summary

Through the literature review it has become clear that stem respiration research involves more than measuring stem E_{CO_2} alone. Chapter 2 gives an overview of the techniques that were applied throughout this thesis to quantify the various CO_2 fluxes in a stem segment and to gain insight into the stem respiration process. When several alternative techniques were applied, both the virtues and shortcomings with respect to stem respiration research were discussed. Particular attention was paid to the techniques for measuring xylem CO_2 concentrations ($[CO_2]$), since some of the techniques have only recently been introduced in tree physiological research. These new techniques proved to hold great promise for gaining insight into the internal CO_2 fluxes in tree stems.

Because the transpiration stream may affect stem E_{CO_2} measurements, we performed the first experiment on a leafless oak and beech, so that sap flow was excluded. Temperature-independent variations in stem E_{CO_2} and xylem $[CO_2]$ were observed, with lower values during the day compared to the night. Explanations found in literature for daytime depressions in stem E_{CO_2} relate to export of respiratory CO_2 by the transpiration stream or to reduced stem R_S because of lower stem water content. However, these explanations failed to explain our results. Although the cuvettes that were used to measure stem E_{CO_2} excluded all light, we suggested that corticular and/or wood photosynthesis may have caused the daytime depressions. Photosynthesis may have reduced the internal $[CO_2]$ in the stem parts adjacent to the cuvette, resulting in an axial CO_2 gradient and, hence, in axial CO_2 diffusion from the enclosed stem segment to the uncovered parts of the stem. It was also observed that drilling a hole in the vicinity of the stem cuvette caused a sharp decrease in E_{CO_2} of the enclosed segment, supporting the idea that the loss of internal CO_2 in the neighbourhood of the cuvette (in this case through removal of CO_2 diffusion barriers) created an axial CO_2 gradient inside the stem and, as a result, axial CO_2 diffusion.

The transpiration stream has often been claimed to cause daytime depressions in stem E_{CO_2} because it is capable of exporting dissolved respiratory CO_2 . However, another consequence of sap flow is the depletion of the water reserves in living stem tissues. Several processes, growth in particular, are known to be very sensitive to water deficit. Water deficit in living stem tissues may reduce stem metabolic activity and thus R_S . However, identification of water deficit in living stem tissues is not easily accomplished. We applied the RCGro model for simulating changes in turgor in the living stem tissues external to the xylem, which are a good indicator for the dynamics in water status. The model also enables to identify when radial stem growth occurs. Variations in temperature-normalized stem E_{CO_2} of a young beech and oak tree correlated fairly well with variations in turgor. Daytime depressions in E_{CO_2} coincided with periods where turgor was lower than the threshold for irreversible growth to occur. However,

even when turgor was still lower than the threshold, E_{CO_2} also responded to changes in turgor, suggesting that also maintenance processes were affected by changes in water status. With the available data we could not distinguish between the two effects of sap flow on stem E_{CO_2} . Both effects may equally well explain the daytime depressions.

In order to further examine the influence of water status on stem R_S , a young oak tree was subjected to soil water depletion under controlled environmental conditions. Before drought, stem E_{CO_2} and xylem $[CO_2]$ correlated very well with stem temperature (T_{st}) and daytime depressions did not occur. Sap flow had no visible effect on stem metabolism. During drought, clear daytime depressions in both stem E_{CO_2} and xylem $[CO_2]$ were observed, which correlated very well with reductions in stem diameter. It is likely that stem E_{CO_2} and xylem $[CO_2]$ were affected by the diurnal dynamics in stem metabolic activity in response to changes in stem water status. When the tree was re-watered, stem E_{CO_2} and xylem $[CO_2]$ increased sharply, most probably because of the improved metabolism of the living stem tissues. A remarkable finding of the experiment is that xylem $[CO_2]$ was not always strictly correlated with E_{CO_2} . This decoupling may be due to large diffusion resistances of xylem and cambium layers and a predominant influence of processes occurring in cambium and phloem tissues on E_{CO_2} .

In a next experiment it was further examined how xylem $[CO_2]$ and stem E_{CO_2} relate to each other through artificially introducing CO_2 enriched water in the stem base of eastern cottonwood trees under controlled environmental conditions. Xylem $[CO_2]$ at approximately 0.5 m above the injection point increased significantly, pointing out that CO_2 was transported with the transpiration stream. The parallel change in xylem $[CO_2]$ and E_{CO_2} indicated that xylem $[CO_2]$ influenced stem E_{CO_2} . The degree of response of stem E_{CO_2} to increased xylem $[CO_2]$ holds information on the resistance of the stem to radial CO_2 diffusion. Although the examined trees were of the same clone, their resistances varied substantially. The large variability in resistances may be an overlooked factor for the large variability in woody tissue E_{CO_2} observed among trees.

In a last experiment the relationship between stem E_{CO_2} and dissolved CO_2 concentration in the xylem sap ($[CO_2^*]$) was investigated in eastern cottonwood trees under different natural weather conditions. Furthermore, stem R_S was calculated with the method developed by McGuire and Teskey (2004) and the relationship between both E_{CO_2} and R_S and T_{st} was investigated. E_{CO_2} correlated generally well with T_{st} , but not during rain and during sunny daytimes. At first sight E_{CO_2} appeared to be decoupled from xylem $[CO_2^*]$, but after removing the temperature effect from E_{CO_2} data, a strong relationship between the two variables was found. Xylem $[CO_2^*]$ dynamics were not related to T_{st} but were

Summary

inversely correlated with sap flow, most probably because sap flow affected the CO_2 transport rate, but a potential influence of sap flow on the water status and, hence, the metabolic activity of the living xylem cells can not be excluded. E_{CO_2} was found to be the largest component of R_S . R_S correlated generally well with T_{st} but not on all times. Particularly on rainy days, R_S was much higher than expected from T_{st} , which could be attributed to an improved cell metabolism because of a better stem water status. However, it is also possible that unknown errors in measurements or in the calculation of R_S contributed to the discrepancy between R_S and T_{st} .

Samenvatting

Uit vroeger onderzoek is gebleken dat respiratie (R_S) één van de sleutelprocessen is die bepalen of bossen netto koolstof afgeven of opslaan (Lindroth et al. 1998, Valentini et al. 2000). Er wordt geschat dat tot een derde van de totale koolstofafgifte door respiratie van boscosecosystemen wordt veroorzaakt door R_S van stammen en takken (Damesin et al. 2002). Ondanks het belang van R_S van houtige biomassa voor de bepaling van koolstofbalansen van boscosecosystemen, is dit proces onvoldoende begrepen. De belangrijkste hinderpaal bij het onderzoek naar R_S van houtige biomassa is het technisch probleem om respiratie te meten. In de meeste studies wordt verondersteld dat R_S van een stam- of taksegment kan worden begroot door het opmeten van de CO_2 efflux (E_{CO_2}) van dat segment. In de meeste studies wordt E_{CO_2} zelfs gebruikt als synoniem voor R_S . Nochtans is er vaak gerapporteerd dat E_{CO_2} van stammen en takken 'onverwachte' patronen vertoont, zoals dagelijkse variaties die niet gerelateerd zijn aan temperatuur, of een extreem grote variabiliteit binnen één boom, tussen bomen of tussen boombestanden. Vandaag slagen wetenschappers er nog steeds niet in E_{CO_2} van houtige biomassa eenduidig te voorspellen of op te schalen. Dit kan te wijten zijn aan onvoldoende begrip van de processen die R_S van houtige biomassa controleren, maar ook omdat E_{CO_2} geen accurate weerspiegeling is van de eigenlijke R_S .

Het eerste hoofdstuk van deze thesis geeft een overzicht van de beschikbare literatuur over de oorsprong en de bestemming van intern CO_2 in boomstammen. Een belangrijke bron van intern CO_2 is de R_S van de levende cellen in de stam. Daarnaast kan ook CO_2 komend van bodemwater of van wortelrespiratie door de sapstroom worden geïmporteerd in de stam om zo de interne CO_2 concentratie te verhogen. Een welbekende bestemming van intern CO_2 is de radiale diffusie naar de atmosfeer (i.e., E_{CO_2}). CO_2 kan echter ook gerefixeerd worden in schors- en/of houtfotosynthese of het kan worden geëxporteerd door de transpiratiestroom. Het hoofdstuk bespreekt de potentiële problemen die geïdentificeerd zijn in vroeger onderzoek bij het gebruik van stam E_{CO_2} als een maat voor stam R_S . Een belangrijk probleem is dat het transport van CO_2 door de transpiratiestroom de relatie tussen E_{CO_2} en R_S kan beïnvloeden. Het hoofdstuk eindigt met een

bespreking van de methode die onlangs werd ontwikkeld (McGuire en Teskey 2004) voor het kwantificeren van de actuele R_S van een stamsegment. Deze methode brengt zowel de externe (i.e., E_{CO_2}) als de interne fluxen van CO_2 in rekening.

Uit de literatuurstudie blijkt duidelijk dat onderzoek naar stam R_S meer inhoudt dan enkel het opmeten van stam E_{CO_2} . Hoofdstuk 2 geeft een overzicht van de technieken die werden toegepast in deze thesis om de verschillende fluxen van CO_2 in een stamsegment te kwantificeren en inzicht te verkrijgen in het proces van stam R_S . Wanneer verschillende alternatieve technieken werden gebruikt, werden de voor- en nadelen met betrekking tot stamrespiratie-onderzoek toegelicht. In het bijzonder werd aandacht besteed aan de technieken voor het opmeten van CO_2 concentraties ($[CO_2]$) in het xyleem omdat sommige technieken zeer recent werden geïntroduceerd in boomfysiologisch onderzoek. Er werd aangetoond dat de nieuwe technieken belangrijke informatie leveren met betrekking tot interne CO_2 fluxen in boomstammen.

Omdat de transpiratiestroom metingen van stam E_{CO_2} sterk kan beïnvloeden, werd het eerste experiment uitgevoerd op een bladloze eik en beuk, zodat sapstroom uitgesloten was. Temperatuursonafhankelijke variaties in stam E_{CO_2} en xylem $[CO_2]$ werden geobserveerd, met lagere waarden overdag dan 's nachts. Verklaringen in de literatuur voor dagdepressies in stam E_{CO_2} hebben betrekking tot de export van gerespireerd CO_2 door de transpiratiestroom of tot een verlaagde stam R_S omwille van een lagere waterinhoud van de stam. Deze verklaringen kunnen de door ons bekomen resultaten echter niet verklaren. Hoewel ondoorzichtige cuvettes werden gebruikt voor het opmeten van stam E_{CO_2} , zijn we van mening dat schors- en/of houtfotosynthese de dagdepressies veroorzaakt kunnen hebben. Fotosynthese kan namelijk de interne $[CO_2]$ in de stam onder en boven de cuvette verlagen, waardoor een axiale CO_2 gradiënt ontstaat die resulteert in axiale CO_2 diffusie van het door de cuvette omsloten stamsegment naar de andere delen van de stam en dus in een verlaagde opgemeten E_{CO_2} . In deze studie werd ook geobserveerd dat het boren van een gat onder de cuvette leidde tot een sterke daling in de opgemeten stam E_{CO_2} , wat de idee ondersteunt dat een daling in de interne CO_2 concentratie in de buurt van de cuvette (in dit geval door het wegnemen van diffusiebarrières voor CO_2) een axiale CO_2 gradiënt in de stam veroorzaakt en leidt tot axiale CO_2 diffusie.

Er wordt vaak beweerd dat de transpiratiestroom dagdepressies in stam E_{CO_2} veroorzaakt omdat het stromend xyleemsap opgelost CO_2 kan exporteren uit het bestudeerde stamsegment. Een ander gevolg van sapstroom is echter de uitputting van de waterreserves in de levende weefsels van de stam. Verschillende processen, waaronder voornamelijk groei, zijn zeer gevoelig voor watertekort. Watertekort kan met andere woorden leiden tot een daling in de

metabolische activiteit van de levende stamweefsels en dus een daling in R_S . De identificatie van watertekort in levende stamweefsels is echter geen sinecure. In deze studie werd het RCGro model gebruikt voor het simuleren van dynamiek in turgor in de levende stamweefsels naast het xyleem, welke een goede indicator is voor de waterstatus. Het model is ook in staat te identificeren wanneer radiale stamgroei optreedt. Variaties in temperatuursgecorrigeerde stam E_{CO_2} van een jonge beuk en eik waren goed gecorreleerd met variaties in turgor. Dagdepressies in stam E_{CO_2} vielen samen met periodes waarin de turgor lager was dan de drempelwaarde nodig voor groei. Zelfs wanneer de turgor lager was dan de drempelwaarde, reageerde E_{CO_2} op veranderingen in turgor, wat doet vermoeden dat ook onderhoudsprocessen beïnvloed werden door veranderingen in waterstatus. Met de beschikbare data was het echter onmogelijk onderscheid te maken tussen de twee effecten van sapstroom op stam E_{CO_2} . Beide effecten kunnen de dagdepressies verklaren.

Om de invloed van waterstatus op stam R_S verder te onderzoeken werd een jonge eik onderworpen aan droogtestress door de bodem te laten uitdrogen onder gecontroleerde omstandigheden. Vóór de droogte waren stam E_{CO_2} en xyleem $[CO_2]$ sterk gecorreleerd met stamtemperatuur (T_{st}) en dagdepressies kwamen niet voor. Sapstroom had geen zichtbare invloed op het metabolisme van de levende stamweefsels. Gedurende de droogte werden duidelijke dagdepressies in zowel stam E_{CO_2} als xyleem $[CO_2]$ geobserveerd, welke sterk gerelateerd waren met reducties in stamdiameter. Het is waarschijnlijk dat stam E_{CO_2} en xyleem $[CO_2]$ beïnvloed waren door de dagelijkse dynamiek in metabolische activiteit van de levende stamweefsels als respons op veranderingen in stamwaterstatus. Wanneer de boom opnieuw werd geïrrigeerd, namen stam E_{CO_2} en xyleem $[CO_2]$ sterk toe, waarschijnlijk omwille van het verhoogd metabolisme van de levende stamweefsels. Een opmerkelijk feit is dat xyleem $[CO_2]$ niet altijd sterk gecorreleerd was met stam E_{CO_2} . De ont koppeling kan te wijten zijn aan de grote diffusieweerstanden van xyleem en cambium en een overheersende invloed van processen in cambium en floëem op E_{CO_2} .

In een volgend experiment werd verder onderzocht hoe xyleem $[CO_2]$ en stam E_{CO_2} met elkaar gerelateerd zijn door het artificieel introduceren van CO_2 verrijkt water in de stambasis van populieren onder gecontroleerde omstandigheden. Xyleem $[CO_2]$, ongeveer 0.5 m boven de injectieplaats, vertoonde een significante stijging, wat erop wijst dat CO_2 door de transpiratiestroom getransporteerd was. De parallelle verandering in xyleem $[CO_2]$ en E_{CO_2} toonde aan dat xyleem $[CO_2]$ een invloed had op E_{CO_2} . De mate van respons van stam E_{CO_2} op verhoogd xyleem $[CO_2]$ bevat informatie over de weerstand voor radiale diffusie van de stam. Hoewel de bomen afkomstig waren van dezelfde kloon, verschilden de weerstanden aanzienlijk. De grote variabiliteit in weerstanden is

Samenvatting

mogelijks een over het hoofd geziene oorzaak voor de grote variabiliteit in houtige biomassa E_{CO_2} tussen bomen.

In een laatste experiment werd de relatie tussen stam E_{CO_2} en de opgeloste CO_2 concentratie in het xyleemsap ($[CO_2^*]$) onderzocht in populieren onder natuurlijke weersomstandigheden. Verder werd stam R_S berekend met de methode ontwikkeld door McGuire en Teskey (2004) en de relatie tussen zowel E_{CO_2} als R_S en T_{st} werd onderzocht. E_{CO_2} correleerde over het algemeen goed met T_{st} , maar niet gedurende regen of op zonnige middagen. Op het eerste zicht leek E_{CO_2} ontkoppeld te zijn van xyleem $[CO_2^*]$, maar wanneer het temperatuurseffect van de E_{CO_2} data verwijderd werd, werd een sterke relatie bekomen tussen de twee variabelen. De dynamiek van xyleem $[CO_2^*]$ was niet gerelateerd met T_{st} , maar was invers gecorreleerd met sapstroom, waarschijnlijk omdat sapstroom de CO_2 transportsnelheid beïnvloedde. Een mogelijks effect van sapstroom op de waterstatus en dus op de metabolische activiteit van de levende xyleemcellen kan echter niet worden uitgesloten. E_{CO_2} bleek de grootste component van R_S . R_S correleerde over het algemeen met T_{st} , maar niet op alle momenten. In het bijzonder gedurende regen was R_S veel hoger dan verwacht op basis van T_{st} , wat te wijten kan zijn aan een verhoogd celmetabolisme door een verbeterde waterstatus. Het is echter ook mogelijk dat ongekende fouten in metingen of in de berekening van R_S bijdroegen tot de discrepantie tussen R_S en T_{st} .

Curriculum vitae

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- 12 Feb - 31 Jun 2002 Universidad Politécnica de Valencia, Valencia, Spain. Erasmus student exchange program

Grants and prizes

- June 2006 Grant from the Research Foundation - Flanders (FWO - Vlaanderen) for the research at the University of Georgia, USA
- July 2003 Ph.D. fellowship from the Special Research Fund (BOF) of Ghent University for the Ph.D. research at Ghent University, Belgium
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- October 2005 Best poster prize for the poster "Analysis of CO₂ efflux rate and xylem CO₂ partial pressure of young tree stems during the dormant season" at the 11th Ph.D. Symposium on Agricultural and Applied Biological Sciences, Leuven, Belgium

Educational activities

- 2003 - 2006 Guidance of practical courses of "Ecofysiologie" and "Plant-Water Relations"
- 2004 - 2005 Guidance of the M.Sc. thesis of Stijn Van Slycken "Stam- en takrespiratie bij beuk (*Fagus sylvatica* L.) in groeikamer- en veldomstandigheden"

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